Trace Greenhouse Gas Fluxes in Upland Forests

Thesis

Trace Greenhouse Gas Fluxes in Upland Forests

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Abstract

Tree stems can act as a conduit for trace greenhouse gases (GHG) produced in the soil. However, the majority of studies describing tree stem fluxes of methane (CH_4) and nitrous oxide (N_2O) have focused on wetland ecosystems. Tree stem fluxes of GHGs on free-draining soils are understudied, but they are assumed to be a source of CH_4 and a weak source of N_2O. The work presented in this thesis aimed to determine how climatic variables, soil abiotic conditions, and tree species influence CH_4 and N_2O fluxes in forests on free-draining soil.

Soil and stem CH_4 and N_2O fluxes were measured in lowland tropical rainforest in Panama, Central America and temperate woodland in the UK, using chambers installed on the forest floor or strapped to individual stems of two common tree species. Air samples were collected every two to four weeks during 5 months in 2014 and during November 2015 at the tropical site, and between February 2015 and January 2016 at the temperate site.

Tree stem CH_4 fluxes differed significantly between species at both sites and stem N_2O fluxes also differed between species at the tropical site. However, there was little variation in soil CH_4 or N_2O fluxes. At both sites, tree-mediated CH_4 fluxes declined from positive values (emission) at the stem base to negative values (uptake) higher up. Stem CH_4 fluxes generally increased significantly with solar radiation, suggesting a link to photosynthetic activity mediated by tree water transport.

Collectively, these results show that trees on free-draining soils could act as net sinks for CH_4 and N_2O. These findings will improve GHG budgets because tree stem uptake is currently unaccounted for. In particular, if uptake of CH_4 by tree stems on free-draining soils is widespread, the global terrestrial CH_4 sink could be much larger than currently estimated.

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Chapter 1: Introduction

1.1 Global CH_4 and N_2O exchange

Methane (CH_4) and nitrous oxide (N_2O) are the second and third most important greenhouse gases (GHGs) after carbon dioxide (CO_2) with radiative effects 28 and 265 times greater than CO_2 (IPCC, 2013). Atmospheric CH_4 and N_2O have increased by 150% and 20% respectively, since pre-industrial times (IPCC, 2013). Despite this, the processes underlying the emission and consumption of CH_4 and N_2O are not yet fully understood, especially in temperate and tropical forests. Much of the research into CH_4 and N_2O emissions to date focuses on rice paddies and wetlands as these are significant sources of both gases. Rice paddies are a major source of anthropogenic CH_4 and contribute heavily to agricultural CH_4 emissions (Kirschke *et al.*, 2013). Natural wetlands are the single largest natural CH_4 source (177-284 Tg CH_4 yr^{-1}; Kirschke *et al.*, 2013) and understanding how they respond to climatic and environmental change will be important to gauge the impact of anthropogenic CH_4. Wetland trees have been identified as sources of CH_4 (Rusch and Rennenberg, 1998; Terazawa *et al.*, 2007; Pangala *et al.*, 2013) and the composition of the gases emitted from the stems reflects the gas concentrations in the soil (Nisbet *et al.*, 2009). Soil CH_4 is produced in anaerobic microsites within the soils with high populations of methanogenic archaea (Teh *et al.,* 2005) However, few studies have examined trace greenhouse gas dynamics in forests on free-draining soils (frequently referred to as upland soils relative to wetlands due to their hydrology). Free-draining, aerated soils are presently estimated to provide a terrestrial CH_4 sink of 9-47 Tg CH_4 yr^{-1} (Ciais *et al.*, 2013).

In contrast to CH_4, the majority of global N_2O is produced in nature however natural emissions have been significantly enhanced by deposition of reactive nitrogen from anthropogenic activity (Templer *et al.*, 2012). Natural N_2O emissions are estimated to be ~10-12 Tg y^{-1}, of which ~6.6 Tg y^{-1} comes from soils under natural vegetation (Davidson and Kanter, 2014). Tropical rainforests are estimated to be the source of 14-23% to global N_2O emissions (Kiese *et al.*, 2005). Temperate forest soils are estimated to contribute between 0.1 and 2.0 Tg N_2O yr^{-1} to the atmosphere, accounting for 0.6 - 11% of total global emissions (Eickenscheidt *et al.,* 2011).

Recently there has been growing interest in tree stem CH_4 and N_2O emissions, particularly as they may contribute the majority of ecosystem CH_4 emissions in tropical wetland forests (Pangala *et al.*, 2013). As free-draining soils cover a much greater land-surface area than wetlands (Pan , even minor contributions to the production, emission or consumption of CH_4 and N_2O from tree stems could have a substantial impact on natural trace gas emissions at the ecosystem and global scales.

1.2 Literature Review

1.2.1 The role of soils in trace gas emissions

1.2.1.a Methane

Soils are the major producers of forest CH_4 through microbial methanogenesis. Clay-rich and highly porous soils help to foster ideal conditions for methanogenesis and are common to tropical rainforests but less common in temperate forests (Vitousek and Sanford, 1986). Methanogenesis is a syntrophic process between microbial consortia (primarily Archaea) because the waste products of each step would accumulate to toxic levels if the entire process was performed by a single species (McInerney and Beaty, 2009). The acetoclastic pathway of methanogenesis involves the breakdown of plant polymers to acetate, which is respired to form CH_4.

Polymers → Monomers → Products → Acetate → CH_4 and CO_2
(Cellulose) (Glucose) (e.g. Acetate Alcohols)

The anaerobic process of methanogenesis begins when pore spaces fill with water, lowering diffusion rates from the atmosphere into the soils and decreasing oxygen (O_2) concentrations (Sposito, 2008). As O_2 availability declines, the rate of root and microbe respiration increases, lowering soil O_2 concentrations which exacerbates anoxic conditions and facilitating CH_4 production (Borken *et al.*, 2006; Teh *et al.*, 2008; Luo *et al.*, 2013). Indeed, there is a strong negative relationship between soil CH_4 concentrations and soil O_2 concentrations (Liptzin *et al.* 2011). The decline in soil O_2 concentrations results in highly reducing conditions, which lead to an increase in pH (Keller and Reiners, 1994). As soils become more acidic, conditions become suboptimal for methanotrophs, reducing rates of methanotrophy and increasing CH_4 emissions (Hanson and Hanson, 1996). Under reducing conditions CO_2 and simple organic compounds can be reduced to form CH_4 via the hydrogentrophic pathway further increasing soil CH_4 concentrations (Liptzin *et al.*, 2011):

$$CO_2 + 8\ H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$

Methanotrophy and methanogenesis in forest soils can be affected by availability of other nutrients in the soil. The ease with which leaf litter is mineralised is controlled by the Carbon:Nitrogen (C:N) ratios in forest soils (Berg, 2000). Soil CH_4 fluxes in a variety of European temperate forests on free-draining soils were positively related to the C:N ratio of the soil (Gundersen *et al.*, 2012). Methane oxidation is largely catalysed by the methane mono-oxygenase enzyme which is able to process ammonium and CH_4. As N availability increases, the competition between the two molecules reduces CH_4 oxidation (Hanson and Hanson, 1996).

As methanogenesis is a biological process, it is subject to metabolic controls and hence the rate of CH_4 production increases with temperature (Gunderson *et al.*, 2012; Inglett *et al.* 2012). Whereas temperatures may not vary much in the tropics, the few degrees of change during the wet season could make a substantial difference to trace greenhouse gas production on a regional scale. As there is greater temperature variability in the temperate zone, soil temperature has a much more significant role in both CH_4 and N_2O fluxes. For example, changes in soil temperature (with time lags of up to two weeks) could explain the majority of temporal variation observed in N_2O and CO_2 fluxes in beech forests in Austria by reducing microbial activity (Kitzler *et al.,* 2005). Between -5ºC and 10ºC, temperature significantly constrains microbial activity in temperate forest soils reducing CH_4 oxidation rates but there was no effect of temperature on oxidation rates between 10ºC and 25ºC (Castro *et al.*, 1995). *In situ* temperature manipulation experiments in a hardwood forest in New York demonstrated that although soil temperature was positively correlated with soil trace GHG efflux, soil moisture content was more important (McHale *et al.,* 1998). Abiotic CH_4 production from temperate soils can be caused by exposure to UV-B radiation or increased temperatures - both of which accelerate degradation of soil organic matter (Jugold *et al.*, 2012). However, this pathway is unlikely to be a significant source of CH_4 globally at this time.

1.2.1.b Nitrous oxide

There are two major natural sources of N_2O: the oceans and soils. In both cases N_2O is produced by nitrification and denitrification. This review focuses on terrestrial N_2O. Globally, emissions of N_2O from soils in natural ecosystems account for between 3.37-6.60 Tg N yr^{-1} (Zhuang *et al.*, 2012), which is estimated as 37% of total global surface emissions (IPCC, 2007). Similarly to CH_4, N_2O is produced by microbes, albeit predominantly by bacteria. Nitrification is an oxidative process and subsequently, it dominates in dry, aerated soils resulting in lower N_2O emissions compared to denitrification, which occurs under the same conditions as methanogenesis (Davidson *et al.*, 2000).

The production of N_2O by nitrification is an aerobic process in which ammonium or ammonia are oxidised to form nitrate that can then be absorbed by plants. N_2O is formed as a by-product of the intermediate stages of this process (Fig.1), primarily during the formation of nitrite, but there is some evidence to suggest that incomplete oxidation of the hydroxylamine could also produce nitrous oxide (Hooper and Terry, 1979).

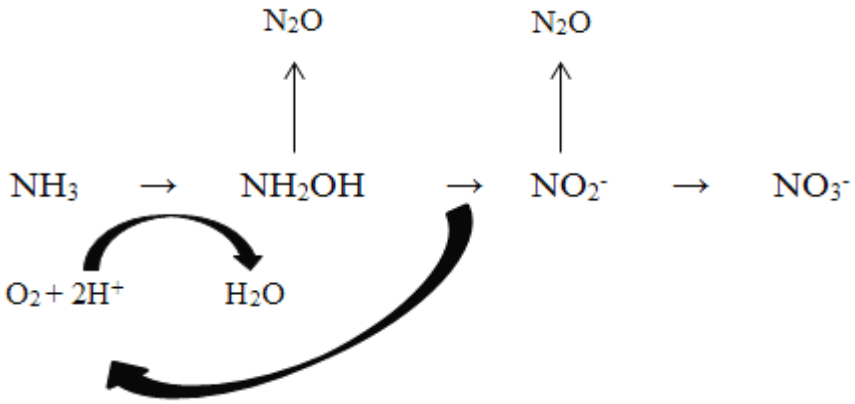

Fig. 1.1 Outline of the process of nitrification. (After Wrage *et al.*, 2001).

Until relatively recently it was assumed that nitrification was solely a bacterial process, yet analyses of microbial DNA and RNA from a variety of soil environments found that nitrification involves both bacteria and archaea (Prosser and Nicol, 2008; Hink *et al.*, 2016). Ammonia oxidizing bacteria (AOB) and ammonia oxidising archaea (AOA) both produce N_2O, however N_2O production is less from AOA than AOB. As AOA are prokaryotes they lack the additional enzymes found in the eukaryotic AOB, NO reductase in particular, which lowers the rate at which they can oxidise ammonia. The yield of N_2O compared to nitrite is 50% lower in AOA than AOB (Prosser and Nicol, 2008; Hink *et al.*, 2016). However, soil pH can affect the activity of each group of ammonia oxidisers, whereby low pH conditions favour AOA; in regions with pronounced dry seasons, this could lead to lower N_2O fluxes from soil surfaces.

Denitrification is the reduction of nitrate to nitrogen gas, completing the nitrogen cycle. N_2O is the penultimate intermediate product in the process and as the diffusivity of gases is much lower in wet, anoxic soils, nitric oxide is reduced to N_2O before it is emitted from the soil. Consequently, N_2O is the dominant end product and greater amounts are released compared to nitrification (Davidson *et al.*, 2000). Similarly to nitrification the amount of N_2O released is governed by pH as well, because lower pH values inhibit N_2O reductase enzymes. The intermediate stages of denitrification are shown below:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Soil water content (SWC) is the primary control of N_2O production. As the water filled pore space decreases and the concentration of O_2 rises, the aerobic metabolism of denitrifying bacteria is promoted over the anaerobic, which lowers the rate of nitrate reduction (Wrage *et al.*, 2001). The optimum SWC for peak N_2O emission is 70-80%, at SWC >80%, N_2 is the end product (Davidson *et al.*, 2000). It has been suggested that in soils where SWC exceeds 80% rapid initialization of strictly anaerobic conditions favours N_2 formation over N_2O formation (Butterbach-

Bahl *et al.*, 2013). Soil N_2O fluxes in mesocosm studies were found to have a bell-shaped response curve to increasing soil temperatures, with the highest rates of N_2O efflux occurring ~20-35ºC (Barnard *et al.*, 2005).

Soil chemistry, in particular pH and soil C:N ratios, can significantly affect rates of denitrification. Forest soil N_2O fluxes are positively correlated with soil pH. Soil pH <6 inhibits the activity of the nitrous-oxide reductase enzyme favouring N_2O production over complete denitrification to N_2 (Šimek and Cooper, 2002). Low C:N ratios increase the availability of N; this in turn increases nitrate concentrations leading to higher N_2O emissions (Gundersen *et al.*, 2012). Denitrification requires labile C as an electron carrier so larger amounts of soil organic carbon could increase soil N_2O fluxes (He *et al.*, 2016). Certainly plant groups, in particular grasses and clovers, that possess nitrogen-fixing root systems can also increase ecosystem N_2O efflux (Skiba *et al.,* 1993).

1.2.2 Emission pathways of CH_4 and N_2O

CH_4 and N_2O can be emitted either directly from the soil itself or through plants. The three abiotic methods of emission from soils are diffusion, ebullition and advection. In waterlogged soils there is no continuous gas phase through which gases can diffuse (Kirk, 2004) therefore diffusion occurs at 10^{-4} times the rate in air filled pore space (Topp and Pattey, 1997). The second abiotic means of emission is ebullition which occurs when gas bubbles form in the soil and are released into the atmosphere when they reach the surface. These bubbles form spontaneously in supersaturated soil water (Kirk, 2004) when the partial pressures of trace gases in water are greater than the hydrostatic pressure at depth (Morel and Herring, 1993). Diffusion and ebullition are observed routes of CH_4 emissions across tropical (Couwenberg *et al.*, 2010), boreal (Ström *et al.*, 2005) and temperate ecosystems (Goodrich *et al.,* 2011). However in free-draining forest soils their role is likely to be secondary to that of plants in general and trees in particular.

Plants provide a biotic means of CH_4 and N_2O emission from deeper soils past the nitrifying and methanotrophic bacteria and archaea that inhabit the upper soil strata. Wetland and riparian plant species, reeds and sedges especially, have evolved structures called aerenchyma which enable them to transport atmospheric oxygen down to roots that are often in highly anoxic soils. Aerenchyma also allow the diffusion of CH_4 and N_2O to the atmosphere (Laanbroek, 2010). Some studies show that plants can produce precursor molecules for CH_4 even under aerobic conditions however the validity of this pathway is still under scrutiny (Keppler *et al.*, 2006; Dueck and van der Werf, 2008) .

1.2.3 Tree stem emissions of CH_4 and N_2O

Trees could represent a major conduit of soil CH_4 and N_2O to the atmosphere. Analysis of satellite near-IR spectroscopy found that models of global methane emissions had underestimated tropical

CH_4 sources, suggesting that biogenic emissions and evergreen rainforest may explain why observed CH_4 emissions were higher than expected (Frankenberg *et al.,* 2005). Stem CH_4 and N_2O fluxes reflected the composition of soil trace GHG concentrations in mesocosm studies of temperate tree saplings and herbaceous plant species (Rusch and Rennenberg, 1998; Wang *et al.*, 2008; Nisbet *et al.*, 2009). This finding that has been confirmed *in situ* for mature tree stems in a Japanese temperate floodplain forest (Terazawa *et al.*, 2007), Indian mangrove swamp (Purvaja *et al.*, 2004) and Indonesian tropical peat forest (Pangala *et al.,* 2013). Trees roots absorb water from soil which contains dissolved CH_4 and N_2O. As the water is transported up the tree stem, CH_4 and N_2O diffuse from the xylem through the stem tissue to the atmosphere via lenticels and other structures that aid gas exchange (Carmichael *et al.*, 2014).

Tree-mediated transport of trace GHGs through tree stems would bypass the oxygenated top soils where the majority of CH_4 oxidation (Teh *et al.*, 2005; Wolf *et al.*, 2012) and more complete denitrification occurs (Koehler *et al.*, 2009a; Wieder *et al.*, 2011). This could explain why in an Indonesian rainforest, tree stem fluxes of CH_4 accounted for 62-87% of the total ecosystem CH_4 flux (Pangala *et al.*, 2013). If that result is representative of the contribution of tree stem CH_4 fluxes in temperate wetlands and forests on free-draining soils then CH_4 fluxes from forests could be far greater than the ~60 Tg CH_4 yr^{-1} currently estimated from global hardwood forests (Rice *et al.,* 2010).

Trees can adapt to cope with flooding-induced soil anoxia in a variety of ways: hypertrophied lenticels (oversized stem pores to increase plant-atmosphere exchange), adventitious roots (commonly grown from the stem to increase oxygen availability) and enlarged aerenchyma (allows more oxygen to reach the roots) (Havens, 1995; Kozlowksi, 1997). The few studies of CH_4 emissions from tropical trees have focused on mangrove species which have evolved pneumatophores to transport oxygen to submerged roots which again aid CH_4 emissions (Purjava *et al.*, 2004). Tree stem CH_4 fluxes in tropical forest are positively related to stem lenticel density (Pangala *et al.*, 2013). Studies of temperate trees such as alders have shown that they use all the above adaptations when in waterlogged soils. This results in increased CH_4 and N_2O fluxes from the trees as there is increased surface area for emission and easier transmission of trace GHGs from soil to atmosphere (Rusch and Rennenberg, 1998; Gauci *et al.*, 2010). Tree species found in forests on free-draining soils may not necessarily have these adaptations but species with a wider geographic range could have them to survive in a variety of biomes. This would in turn possibly influence rates of stem trace GHG efflux from these forests.

Other aspects of tree physiology, besides adaptation to survive in waterlogged and anoxic soils, can influence gas fluxes from tree stems. These include stem diameter and wood specific density. Tree stem CH_4 fluxes were negatively related to both stem diameter and wood specific density in a tropical peat forest (Pangala *et al.*, 2013). As trees age, stems become thicker, gases must diffuse through more tissue before reaching the atmosphere. With fewer aerenchyma stem

tissue becomes denser retarding stem trace GHG fluxes as it takes longer for gases to diffuse through the stem and out to the atmosphere (Bhullar *et al.*, 2013).

Previous studies in the Amazon (Pangala *et al.*, 2013), China (Wang *et al.*, 2016) and the USA (Megonigal *et al.*, 2016) found decreasing CH_4 fluxes with sampling height across multiple species, but they did not demonstrate stem uptake of CH_4. No relationship has thus far been observed between leaf fall and significant changes in stem CH_4 fluxes in temperate species which would suggest that stem trace GHG efflux is largely driven by passive diffusion (Pangala *et al.*, 2015). This could also explain why fluxes decline with height as the concentration gradient between internal stem concentrations of CH_4 (and N_2O) and atmospheric concentrations becomes smaller.

1.2.4 Biotic controls of CH_4 and N_2O fluxes

As well as acting as conduits for CH_4 and N_2O, trees provide much of the source material for methanogenesis, nitrification and denitrification through root exudates and litterfall (Ding and Cai, 2003; Koelbener *et al.*, 2010). Variation in fine root biomass, litter mass and soil nitrate concentrations can all influence soil N_2O and CH_4 fluxes under different tree species which could lead to comparable effects on stem CH_4 and N_2O fluxes (Wang *et al.*, 2013). Soil organic matter is the main control of soil pH and C:N ratios, much of which is provided by litterfall. Modification of the availability of anions in the soil by trees will affect soil pH, however these effects are limited to the upper 0-10-cm of soils (Augusto *et al.*, 2002; Hagen-Thorn *et al.*, 2004). The ease by which litter is decomposed and mineralised to produce acetate and nitrate will vary between species depending on factors such as leaf lignin content.

Litter quantity can affect nutrient availability for soil microbial communities with associated effects in soil-atmosphere exchange of greenhouse gases. Litter manipulation experiments are used to examine the effects of enhanced net primary productivity (NPP) under future high CO_2 atmospheric conditions and the associated increase in litterfall on soil biogeochemical processes. Litter quantity can influence the rates of GHG emissions from forest soils as it provides acetate used by acetoclastic methanogens and nitrate used in denitrification (Teh *et al.*, 2008). Two key nutrients for plant growth, nitrogen and phosphorus, are cycled for the most part through litterfall (Vitousek, 1982). Hence the nitrogen cycle will be controlled to some extent by the amount of litterfall. A long-term litter manipulation experiment in Panama found soil nitrate concentrations were significantly lower in litter removal plots compared to controls; correspondingly nitrate concentrations were significantly higher in litter addition plots (Sayer & Tanner 2010). It is therefore reasonable to assume that nitrification and denitrification will increase with the amount of substrate and hence N_2O emissions could also rise. The potential link between litter inputs and GHG emissions from the soil was explored further by a study of soil N_2O emissions from a lowland wet forest in Costa Rica, in which doubling leaf litter inputs increased

rates of soil N_2O emissions by 43% relative to controls, with a corresponding decline of 42% in litter removal plots (Wieder *et al.*, 2011). N_2O efflux is generally higher from broad-leaved forests compared to coniferous forests (Ambus *et al.,* 2006) because the slower decomposition of coniferous litter reduces nitrate availability in the soil (Venterea *et al.,* 2003; Ambus *et al.,* 2006; Eickenscheidt *et al.,* 2011 and Fender *et al.*, 2013). Litter manipulation effects on tree stem CH_4 and N_2O emissions are currently unknown, but as the above studies found that changes in mineral soil chemistry from litter were the primary driver of changes in fluxes, it is conceivable that litter manipulation could also affect stem emissions.

Biological activity from other sources could affect tree stem fluxes of CH_4 and N_2O in temperate and tropical forests. Termite activity is estimated to account for 11 Tg CH_4 y^{-1} (Kirschke *et al.*, 2013) and it is possible that a termite nest within the soil or a tree stem could elevate stem trace GHG fluxes in excess of what is otherwise typical for this ecosystem. Heartwood rot is not fully understood but may be more prevalent than we think, and laboratory analyses of tree cores extracted from mature tree stems in temperate upland forests in the USA and China found that heartwood rot may increase GHG fluxes from tree stems (Covey *et al.*, 2012; Wang *et al.*, 2016).

1.3 Statement of the Problem

Research on trace gas fluxes from wetland soils is extensive but there is a gap in our knowledge about forests on free-draining soils. Crucially, given the large variation in CH_4 and N_2O fluxes from soils depending on the season and associated climatic conditions and the uncertainty around tree stem trace GHG fluxes, we have yet to truly determine whether forests on free-draining soils are sinks or sources of greenhouse gases. Furthermore, the scale of trace GHG flux measurements is also a factor. Current estimates of source or sink strength for trace GHGs are based on data from soil CH_4 and N_2O flux measurements and canopy towers, which are in turn used to model CH_4 and N_2O fluxes on an ecosystem or regional scale. However this approach leads to an underestimation when contrasted with satellite measurements (Frankenberg *et al.* 2005). There was a 40% difference between observed and modelled CH_4 concentrations because models failed to account for stratospheric CH_4 and 'unconsidered sources' (i.e. trees) which were strongly correlated with broadleaf evergreen forest cover. In order to better understand the exchange of CH_4 and N_2O between the biosphere and atmosphere, it is vital that tree stem CH_4 and N_2O fluxes are included. This is especially important as tree stem trace GHG emissions from forests on free draining soils could potentially offset the current estimated CH_4 sink for forest soils, turning these ecosystems from minor net CH_4 sinks to net CH_4 sources.

1.4 Hypotheses

The research presented in this thesis aimed to determine the relative contributions of tree stems and soils to ecosystem fluxes of CH_4 and N_2O in tropical and temperate forests on free draining soils. Further, I investigated the impacts of litter and nutrient inputs, seasonal change and species composition to test the following hypotheses:

1) Fluxes of CH_4 and N_2O from forest soils have been shown to vary seasonally in temperate and tropical forests. As tree stem fluxes of CH_4 and N_2O are thought to reflect soil trace GHG concentrations, tree stem fluxes of both CH_4 and N_2O will show significant seasonal variation in temperate and tropical forests.

2) Tree stem CH_4 and N_2O fluxes in wetland forests decreased significantly with stem sampling position above the forest floor. Tree stem trace GHG fluxes in forests on free-draining soils will also decrease significantly with sampling position above the forest floor.

3) Stem fluxes of CH_4 and N_2O can vary due to differences in physiology, wood specific density, root structure and root exudate effects on methanogenesis and denitrification in the surrounding soil. Stem fluxes of CH_4 and N_2O will be greater from canopy species in tropical rainforests and in faster growing temperate species as they will require greater amounts of water to be transported to the leaves to aid photosynthesis.

4) Litter manipulation experiments have shown that litter addition significantly increased rates of soil respiration, nitrate availability and methanogenesis. Consequently tree stem and soil fluxes of CH_4 and N_2O will be greatest in litter addition plots and lowest in litter removal plots.

1.5 Thesis structure

Chapter 2 will explain the characteristics of the field sites in Panama and the UK, the analytical methods utilised in the laboratory and the statistical analyses used to process the data. **Chapter 3** explores the seasonal variation in soil and tree stem fluxes over the dry-to-wet season transition at a long-term litter manipulation in Panama. **Chapter 4** presents an annual dataset of tree stem and soil CH_4 and N_2O fluxes from a temperate woodland in the UK. **Chapter 5** is a snapshot of spatial variation in CH_4 in tropical and temperate forests on free-draining soils and a discussion of the spatial interplay between CH_4 emission and uptake along tree stems. Finally **Chapter 6** gives a discussion of trace greenhouse gas fluxes from the forests on free-draining soils studied together with the global implications of findings from Chapters 3-5 .

Chapter 2: Methods

2.1 Field Sites

2.1.1 Gigante Peninsula, Panama

The Barro Colorado Nature Monument was created in 1923 and includes the island of Barro Colorado (BCI) in the Panama Canal and the surrounding peninsulas. The mean annual temperature at the weather station on BCI is 26ºC, mean annual rainfall is 2,600mm and there is a strong dry season from mid-December to mid-April (Leigh, 1999). The soil in the plots is characterised as a moderately acidic Oxisol (Dieter *et al.*, 2010; Turner & Wright, 2014), with a pH 5.0-5.5 (Cavelier, 1992). Sampling of the uppermost 10-cm of the soil revealed low concentrations of phosphate and potassium, moderate concentrations of inorganic nitrogen and high calcium and magnesium concentrations (Yavitt *et al.*, 2009). All measurements were made within the Gigante Litter Manipulation Project (GLiMP; Fig. 2.1), approximately 5 km south of BCI, Panama, Central America (Fig. 1). The 15 GLiMP plots were set up between 2000 and 2002; each plot measures 45m x 45m and the edges of the plots were trenched to a depth of 0.5-m, lined with plastic and then backfilled (Sayer and Tanner, 2010).

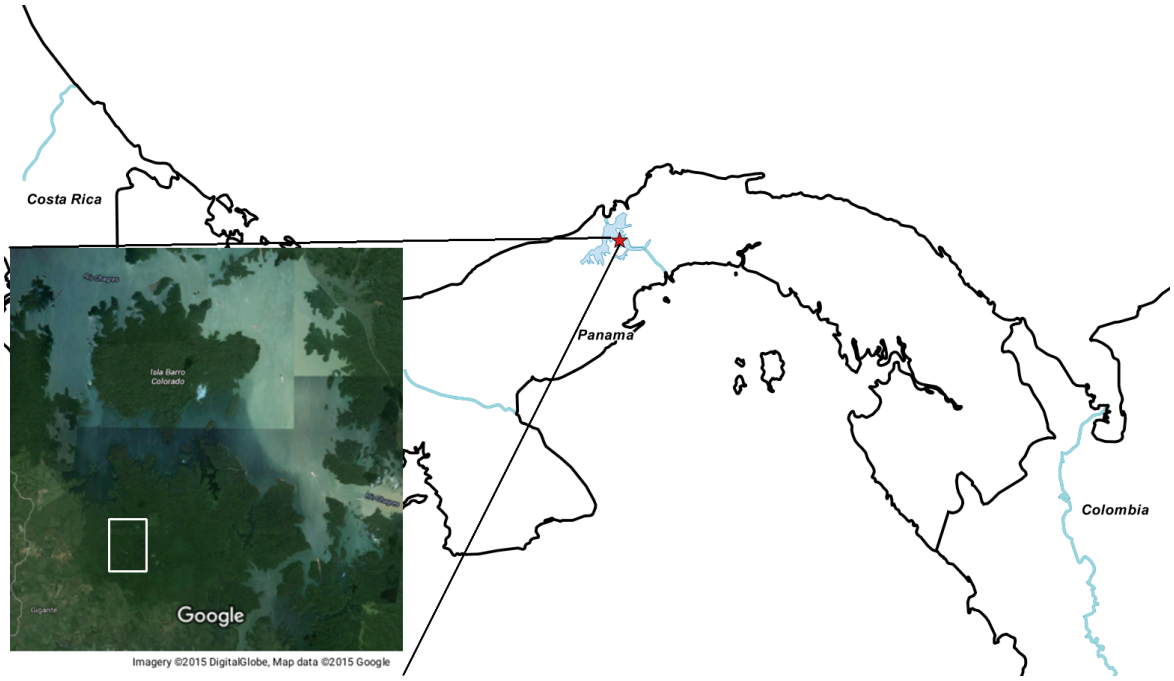

Figure 2.1 Map of Panama with Barro Colorado Island (BCI) marked. Satellite image shows BCI and the Gigante Peninsula to the south (Image from Google Earth). The Gigante Litter Manipulation (GLiMP) experiment is within the area marked by the white box.

Starting in January 2003, the litter is raked up and removed from five plots (L-) and added to five plots where it is spread as evenly as possible (L+); five plots were left as controls (CT). All trees with a diameter >10-cm at breast height (1.30-m) in the plots were measured, tagged and the point of measurement permanently tagged with a horizontal paint line. A full description of the experiment is given in Sayer *et al.*, (2006).

I selected two common tree species for this study: the fast-growing canopy tree *Simarouba amara* (Aubl.) and the shade-tolerant subcanopy tree *Heisteria concinna* (Standl.). Both species have relatively smooth bark and straight stems which facilitates sampling. Trees were mapped and marked using handheld GPS. One individual per species was chosen per plot but only 13 of the 15 experimental plots contained live mature individuals of *Simarouba.* Hence the present study included trees in four plots per treatment, making 12 *Heisteria* and 12 *Simarouba* trees in total. Tree stem and soil gas fluxes were sampled biweekly over the 2014 dry-to-wet season transition, March to August 2014, and 18th - 26th November 2015.

2.1.2 Wytham Woods, United Kingdom

Wytham Woods is a mixed deciduous forest in Oxfordshire, United Kingdom, comprising patches of ancient or old-growth woodland and more recent plantations. My study was established in the 'ForestPrime' litter manipulation plots within a large area of area old growth (*c*. 120-years) mixed woodland Ash (*Fraxinus excelsior* L.), Beech (*Fagus sylvatica* L.), Sycamore (*Acer pseudoplatanus* L.) and Oak (*Quercus robur* L.*;* Fenn *et al.* 2014). The ForestPrime experimental design largely replicates the Gigante Litter Manipulation Project (Sayer *et al.*, unpublished data): 15 experimental plots, each measuring 25-m × 25-m, were established in five blocks in 2013. All plots were trenched to a depth of 0.5-m to limit the transport of nutrients by root and hyphal networks, one side of the trenches was lined with plastic and the trenches were then backfilled. All trees with a diameter at breast height (dbh) >10-cm were measured, tagged and the point of measurement permanently tagged with a horizontal paint line.

Figure 2.2 Map of the United Kingdom with Wytham Woods marked. Satellite image shows the extent of Wytham Woods (Image from Google Earth). The ForestPrime experiment is sited within the area marked by the white box in the north of the woods.

Manipulation of litter within each block commenced in December 2013: in the five L- plots, litter is hand-raked twice a year at times of peak litterfall (October to January); the litter is then spread as evenly as possible over five L+ plots leaving five undisturbed controls. In each plot, four permanent soil collars were installed on each side of the inner 10-m × 10-m of each plot. The collars were made of polypropylene tubes (120-mm height, 200-mm internal diameter), which were sunk into the soil to 30-mm depth. The collars were installed a year prior to the start of measurements and live vegetation was carefully removed from the collars at regular intervals to ensure only litter and dead organic material remained in the collars.

For my study, I only used the control plots and I selected two species that were common to four of the plots: Sycamore (*Acer pseudoplatanus*) and Ash (*Fraxinus excelsior*), as no individuals of Sycamore were present in one of the plots. Measurements were made over the four soil collars and on three individuals of each species in each plot. Tree stem and soil gas fluxes were initially sampled monthly from the 16th February to 14th April 2015, then bi-monthly from 11th May to 20th October 2015 and then monthly again from 3rd November 2015 to 5th January 2016.

2.2 Field Sampling Methods

2.2.1 Common sampling methodologies

Gas fluxes from tree stems were measured by securing a flexible chamber (Siegenthaler *et al.*, 2016) made from a 450-mm × 300-mm sheet of polycarbonate (Bay Plastics Ltd, North Shields, UK), lined with 19-mm wide by 25-mm thick neoprene foam (Seals+Direct Ltd, New Milton, UK). The chambers were attached to the tree stems using cambuckle straps, wrapped around the tree trunk in such a way as to seal the chamber at the top, middle and base (Fig. 2.3). Air samples from within the chamber were extracted through a rubber bung placed into a central sampling port on the chamber at set time intervals which varied between the Panamanian and UK sites.

Figure 2.3 Photograph of tree stem trace greenhouse gas sampling chamber strapped to the stem of a *Heisteria concinna* individual in a lowland tropical rainforest, Panama, Central America. Image taken by author.

Soil greenhouse gas flux samples were taken by syringe after 0, 3, 6 and 10 minutes and injected into pre-evacuated 12-ml borosilicate vials (Exetainers®, LabCo Ltd, High Wycombe, UK). Gas samples from the soil were taken by placing a PVC lid with an inner seal of gas-tight neoprene foam on top of the pre-installed soil collars (Fig. 2.4). Integrity of the seal on the soil chamber was demonstrated by suction when removing the lid after sampling.

Figure 2.4 Photograph of soil trace greenhouse gas sampling chamber on a free-draining lowland tropical rainforest soil, Panama, Central America. Image taken by author.

Air pressure and temperature outside the stem and soil chambers were recorded at the time of closure using a Commeter C4141 Thermometer-Hygrometer-Barometer probe (Comet Systems, Czech Republic) and soil temperatures at 0-10-cm depth adjacent to the soil chambers and tree stems were recorded using a Thermapen (ETI Ltd, Worthing, UK).

2.2.2a Gigante Peninsula, Panama

Greenhouse gas fluxes from the soil were measured using permanently installed soil collars located 2-3 m to the north and south of each tree. The collars were made from 120-mm long sections of polyvinyl chloride (PVC) pipe (internal diameter 200 mm), which were embedded 30-mm into the soil. All collars were installed at least two weeks prior to sampling in March 2014 and an appropriate amount of litter was placed into the collars in the CT and L+ plots to achieve consistency with the surrounding forest floor. To determine CH_4 and N_2O emissions from the soil, a PVC lid with an inner seal of gas-tight neoprene foam was placed on top of the collar; a 15-ml air sample was taken by syringe via a septum in the lid immediately after closure and then again after 3, 6 and 10 minutes. Each sample was injected into pre-evacuated 12-ml borosilicate vials (Exetainer™, LabCo Ltd, High Wycombe, UK). The suction when removing the lid after sampling demonstrated the integrity of the seal on the soil chamber. Soil temperature at 0-6 cm depth was recorded adjacent to the collars using a Thermapen (ETI Ltd, Worthing, UK).

Two common tree species were selected for this study: the fast-growing canopy tree *Simarouba amara* (Aubl.) and the shade-tolerant subcanopy tree *Heisteria concinna* (Standl.). Both species have relatively smooth bark and straight stems, which facilitates sampling. Trees were

mapped and marked using handheld GPS. One individual per species was chosen per plot, but only 13 of the 15 experimental plots contained live mature individuals of *Simarouba*; hence the present study included trees in four plots per treatment, making 12 *Heisteria* and 12 *Simarouba* trees in total. Only 13 of the 15 experimental plots contained live mature individuals of *Simarouba*, hence the present study included trees in four plots per treatment.

The chambers were secured to the tree stem at a height of 0.1-0.3-m for the 2014 and 2015 campaigns (with additional heights at 0.75-m, 1.30-m and 2.00-m in 2015) using cam buckle straps. Gas samples were taken by syringe at 0, 5, 10 and 15 minutes to account for chamber size, and injected into vials as described above. Collection of soil temperature and soil water content data during gas sampling was limited to 28 May - 14 June 2014 and 2 - 6 July 2014 due to equipment malfunction. Consequently, the values presented here were collected monthly from the plots as part of the long-term monitoring of the litter manipulation experiment (Brechet *et al.* unpublished data). Soil temperature at 0-10-cm depth was measured adjacent to the collars using a soil temperature probe and volumetric soil water content at 0-6 cm depth was measured using a Thetaprobe (Delta-T Devices, Cambridge, UK) calibrated to local soil conditions following the manufacturer's instructions. Solar radiation data is provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute in 15-minute intervals, measured on a meteorological tower on BCI at 48-m height using a LiCor LI200X pyranometer (LiCor, Nebraska, USA). The daytime data were modified in R to provide a weekly mean solar radiation value.

Air samples were analysed within a week of sampling to establish CH_4 concentrations. CH_4 in the samples was analysed using off-axis Integrated Cavity Output Spectroscopy (FMA-200 Fast Methane Analyser; Los Gatos Research, Mountain View, CA, USA), modified to employ the 'closed loop' principle (Baird *et al.* 2010). Air samples were returned to the Open University for analysis to determine N_2O concentrations in December 2014. N_2O in the samples was analysed using gas chromatography (Ai 94 Gas Chromatograph, Cambridge Instruments (Ellutia UK), Ely, UK).

2.2.2b Wytham Woods, United Kingdom

After the initial experiment to study trace greenhouse gas dynamics in tropical rainforests on free-draining soils, I decided to perform a companion study at a temperate site in the UK. This experiment was designed to continue investigating the temporal variation of greenhouse gas fluxes from forests on free-draining soils and see what the more pronounced annual temperature variations and variations in aboveground tree activity would have on the gas fluxes.

Soil gas fluxes were measured from four permanently installed soil collars, located along each side of the inner 10-m × 10-m of each plot. The collars were made from 120-mm long sections of polypropylene pipe (internal diameter 200-mm), which were embedded 30-mm into the

soil. 20-ml air samples were taken by syringe after 0, 3, 6 and 10 minutes and injected into pre-evacuated vials. Similarly to the Panama experiment, seal integrity on the soil sampling chambers was demonstrated by lid suction after the 10 minute sampling period had elapsed..

Tree stem CH_4 and N_2O fluxes were sampled using the chamber design outlined in Siegenthaler *et al.* (2016). 20-ml air samples were taken by syringe after 0, 3, 6 and 10 minutes and injected into pre-evacuated vials. The chambers were secured to the tree stem using cam buckle straps. Stem gas fluxes were sampled at 0.3-m, 0.75-m and 1.3-m throughout the experiment. From October 2015 to January 2016, tree stem fluxes were also sampled at 2-m height, after preliminary data from another study showed the potential importance of sampling higher up the stems (Wang *et al.*, 2016). Play-Doh (Hasbro, United Kingdom) was used to seal fissures in Ash bark and bind the chamber to the flaky Sycamore bark, as it ensures a good seal and does not produce or absorb either of the gases studied (Siegenthaler, unpublished data). Sampling times at the UK site were reduced compared to the Panama experiment as 10 minutes was found to be sufficient closure time to generate a measurable flux.

Volumetric soil water content at a depth of 0-6-cm depth was measured monthly using a Thetaprobe (Delta-T Devices, Cambridge, UK) calibrated to local soil conditions following the manufacturer's instructions. Data for monthly mean solar radiation and total rainfall were collected at the weather station in Wytham Woods (UK Environmental Change Network).

All samples were analysed within two weeks of collection. The CH_4 content of the samples was analysed using a Los Gatos Research FMA-200 Fast Methane Analyser (FMA; Los Gatos Research, Mountain View, CA, USA), modified to employ the 'closed loop' principle (Baird *et al.* 2010). The N_2O content of the samples was analysed using a Gas Chromatograph (Ai 94, Ellutia UK – formerly Cambridge Instruments, Ely, UK) fitted with an Electron Capture Detector.

2.3 Laboratory Analyses

2.3.1 Methane

Methane samples were analysed using an FMA-200 Fast Methane Analyser (FMA; Los Gatos Research, Mountain View, CA, USA). The FMA was modified to employ Baird *et al*'s (2010) 'closed loop' principle whereby samples are injected into the device through an injection loop which can then be opened to vent the machine and return the readings to a baseline measurement. The instrument uses off-axis Integrated Cavity Output Spectroscopy (ICOS) and consists of a near-infrared diode, a reflective mirror-lined cavity acting as an absorption cell and a photo-detector. A collimated laser of wavelength 1653.723-nm is beamed at a slight angle and is reflected into the optical cavity which creates a relative path of ~2,500-m. The fractional absorption of the laser at the CH_4 wavelength is recorded by the photo-detector providing the measure of CH_4 concentrations in the cavity. CH_4 reduces the laser beam intensity decay rate as a result of absorption. For each

sample, 4-ml of gas was drawn from the vials by syringe and injected into a closed injection loop attached to the FMA.

2.3.2 Nitrous Oxide

Nitrous oxide was analysed using a Cambridge Instruments (now trading as Ellutia UK) Ai 94 Gas Chromatograph (GC) fitted with an Electron Capture Detector (ECD). The GC is equipped with separate loop sampling and pre-column backflush channels to vent sample into an ECD and flame ionisation detector for the measurement of N_2O and CH_4 respectively. Both channels comprise a back-flush column and an analytical column maintained at a constant temperature of 50^oC in the main oven. The ECD column specifications are:

Column 1 (backflush) – Poropak Q 50 – 80 mesh (max. temp. 250^oC)

Column 2 (analytical) – Poropak Q 80 – 100 mesh (max. temp. 250^oC)

A ten-port valve with integrated heated (50^oC) 2-ml sample loop controls gas flow for each channel, ensuring that the injected sample's temperature matures that of the column. 4-ml of sample gas was loaded into the loops by a fixed syringe before an initial valve change (after 10 seconds) mixes it with a carrier gas into the backflush and analytical columns. After 2 minutes, a second valve change reverses the carrier-gas through the backflush to prevent less-volatile, slower molecular species reaching the detectors. The N_2O peak is detected by the ECD 3 minutes after initial injection.

The resulting chromatograms of signal in millivolts against retention time in minutes show two distinct peaks at 1 minute for CH_4 and 3 minutes for N_2O. Concentrations are proportional to peak height and area. Varian Star software (Agilent Technologies) was used to integrate the peak height and area by defining the baseline and peak parameters for each chemical species. Peak heights are fitted to the linear regression equation of the peak heights of three known concentration standards of 1-ppm, 0.2-ppm and 0.05-ppm N_2O providing the concentration of N_2O in that sample.

2.4 Statistical analyses

2.4.1 Gas flux calculations

The rate of change in CH_4 and N_2O within chambers was calculated from least squares linear regression analysis of concentrations against time taking into account the measurement surface area (i.e. stem or soil) in metres squared and the internal chamber volume in cubic metres. The gas volumes were corrected for air pressure (in kPa) and air temperature (in K) at the time of sampling

to ensure that correct molar concentrations were used to calculate the mass in mg of the gas species at that sampling point using the Ideal Gas Law:

$$n = \frac{PV}{RT} \qquad \text{(Equation 2.1)}$$

Where n is the number of moles for a given gas, P is atmospheric pressure, V is the volume of the chamber, R is the ideal gas constant and T is temperature in Kelvin. Regressions with an $R^2 \geq 0.7$ were used for analysis. Alm *et al.* (2007) (cited in Cooper *et al.*, 2014) noted that low fluxes (especially those near to zero) tend to have low R^2 values and therefore should not be excluded from analysis simply due to a lower coefficient of determination. Gas fluxes were multiplied by 86400 to convert them from mg m^{-2} s^{-1} to mg m^{-2} d^{-1} for initial analyses before being converted to µg m^{-2} h^{-1} for final analyses. This two conversion approach was used to give initial analysis to guide both sampling and statistical processes before conversion to the most commonly used units found in the literature.

As is common with trace GHG sampling (Alm *et al.*, 2007), not all CH_4 flux data measured in Panama and the UK were linear. In cases where one of the four measurements of CH_4 concentration did not fit the linear regression, the CH_4 flux was determined from the three remaining concentration values. All soil CH_4 fluxes were assumed to be diffusive in nature. Ebullition can be a source of soil CH_4 efflux (due to its potential to bypass methanotrophs in the aerated top soil) but extensive spatial and temporal sampling is required to identify whether soil CH_4 fluxes are diffusive or due to ebullition, and this was beyond the scope of the present study..

2.4.2 Data analyses

Greenhouse gas flux data often features a small number of extreme outliers; these values are not necessarily due to measurement error, but may be the result of e.g. biological activity such as termites or heartwood rot (Megonigal *et al.*, 2008; Covey *et al.*, 2012) which could obscure patterns due to tree species identity or climatic variables. Consequently, the data were inspected visually and extreme outliers that lay outside of the 5^{th} - 95^{th} interquartile range were removed. For the 2014 Panama campaign, 20 out of 201 CH_4 tree stem fluxes were removed and 2 out of 188 soil chamber CH_4 fluxes were removed. 3 of 115 tree stem N_2O fluxes were determined to be outliers and 1 flux value for soil chamber N_2O of 108 was outside the 5^{th}-95^{th} interquartile range. Four outlier values were removed from the 153 tree stem CH_4 fluxes measured during the 2015 Panama sampling campaign. For the UK dataset, 6 out of 1100 CH_4 tree stem fluxes were removed and 3 out of 227 soil chamber CH_4 fluxes were removed. 14 of 632 tree stem N_2O fluxes were determined to be outliers and 7 of 211 fluxes for soil chamber N_2O were outside the 5^{th}-95^{th} interquartile range.

All statistical analyses were conducted with and without outliers and full results of the analyses including extreme outlier values are given in Appendices I and II.

All data analyses were conducted in R 3.3.2 (R Core Team, 2016) using the lme4 package for mixed effects models (Bates *et al.*, 2015). Gas fluxes were calculated for each chamber following Baird *et al.* (2010), whereby the least squares linear regression slope of the four sample concentrations is plotted against sampling time and the slope to give the gas flux in $\mu g\ m^{-2}\ h^{-1}$. Gas flux measurements were only used for further statistical analysis if the R^2 of the regression was >0.7; this cut-off point was chosen following Alm *et al.* (2007; cited in Cooper *et al.*, 2014), who noted that low fluxes (especially those near to zero) tend to have low R^2 values.

2.4.2.a Panama mixed effects models

Effects of climatic variables on soil and stem GHG fluxes were assessed using linear mixed effects models (*lmer* function), with air temperature, precipitation and their interaction as fixed effects, and plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and p-values to check for model improvement (Pinheiro and Bates 2000). Having identified which climatic variables to include as covariates, tree species, litter treatment and their interaction were added as fixed effects. These nested models were then compared as above to arrive at a new minimum adequate model. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. The final model fit was inspected using diagnostic plots. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ but due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

Prior to using the same analyses as above for the 2015 campaign tree stem trace GHG data, the significance of variation between CH_4 flux and sampling height was examined using linear mixed effects models (*lmer* function), with sampling height, species and their interaction as fixed effects, and with plot and time as random effects. As sampling height significantly influenced stem GHG fluxes, the data were subsequently analysed for each sampling height separately.

2.4.2.b Wytham Woods mixed effects models

Co-linearity between environmental variables was assessed using linear models and diagnostic plots prior to modelling. The relationships between CH_4 or N_2O fluxes and climate variables were also assessed for co-linearity by calculating the variable inflation factor (VIF; *vif* function in the car package; Fox and Weisberg., 2011). As all variables had a VIF value <2, co-linearity was not

deemed to be an issue for interpreting subsequent models (Zuur *et al.* 2010). Linear models were then used to examine relationships between GHG fluxes and climatic variables. Climate variables that explained a significant proportion of the variation in GHG fluxes were included as covariates in subsequent models.

The effect of sampling height and tree species on stem GHG fluxes was assessed using linear mixed effects models (*lmer* function), with sampling height, species and their interaction as fixed effects, and with plot and time as random effects. As sampling height significantly influenced stem GHG fluxes, the data were subsequently analysed for each sampling height separately.

Effects of climatic variables and their interaction on soil and stem GHG fluxes were assessed using linear mixed effects models (*lmer* function) as fixed effects, and with plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and p-values to check for model improvement (Pinheiro and Bates 2000). Tree species was added as a fixed effect to reach a new minimum adequate model. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. The final model fit was inspected using diagnostic plots. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ but due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

2.4.3 Pivot Points

Significance of spatial variation on stem CH_4 fluxes from the 2015 Panama campaign and October 2015 to January 2016 in the UK was assessed using linear mixed effects models (*lmer* function), with sampling height as a fixed effect, and plot and time as random effects. Once a significant height effect was identified, pivot points (i.e. the height at which stem fluxes are 0 $\mu g\ m^{-2}\ h^{-1}$ and switch from CH_4 sources to sinks) could be calculated. Pivot points were calculated from least squares linear regression of the four stem flux values per tree per sampling date plotted against sampling height whereby the pivot point is equal to the negative of the y-intercept divided by the gradient of the regression slope.

2.4.4 Ecosystem upscaling

Stem fluxes were estimated over the bottom 2.5-m and 15-m (the average canopy height is 15-30-m in moist tropical deciduous forest; Pan *et al.*, 2013 and at Wytham Woods is 15-18-m; Herbst *et al.*, 2007) of tree stems in Panama and Wytham Woods, using equations obtained from the fluxes of

the same species within the same plot. In Wytham, as CH_4 fluxes were not sampled at 2-m stem height until October 2015, CH_4 fluxes at 2-m were estimated for the missing months by deriving an equation using the October 2015 to January 2016 data. Again this was done per species per plot. 2014 dry season stem fluxes for each tree in Panama were estimated using the equations derived from the wet season measurements in 2015.

A species-specific equation was derived from the relationship between stem CH_4 flux and sampling height for each plot to estimate the CH_4 fluxes at heights that were not sampled from. Similarly surface area at the stem sections not sampled was estimated from the surface areas calculated from the stem diameter measured at each sampling height. Surface area at each stem height was multiplied with stem CH_4 flux for that corresponding stem height to obtain fluxes. Initially this was done per individual tree sampled and subsequently all tree CH_4 fluxes were pooled together to estimate a CH_4 flux per tree for the ecosystem in grams per hectare per day. This CH_4 flux value per tree was multiplied with the number of trees in a hectare to get tree emissions per hectare. To estimate CH_4 fluxes along the length of the trees, from a portion of 2.5 to 15 m of the stem height fluxes measured at 2.2-2.5 m was applied. Surface area was estimated using the above equation. Surface area was multiplied with CH_4 fluxes and later emissions per tree was estimated as explained above and applied to a hectare of area. Soil surface area was estimated by subtracting tree basal area from a square hectare and soil CH_4 fluxes were multiplied with this surface area to obtain soil CH_4 fluxes per hectare also in grams per hectare per day.

Chapter 3: Tree stems in a lowland tropical forest on free-draining soil are sources of CH_4 and N_2O

Abstract

- **Tree stems can act as a conduit for trace greenhouse gases (GHGs) produced in the soil. However, the majority of studies describing tree stem fluxes of methane (CH_4) have focused on wetland ecosystems and only a handful of mesocosm studies have reported nitrous oxide (N_2O) fluxes from trees. Tree stems in tropical peat forests emit 60-80% of total ecosystem CH_4 but tree stem fluxes of CH_4 and N_2O on free-draining tropical soils have not been examined.**
- **Tropical forests on free-draining soils are assumed to be a CH_4 sink and a weak source of N_2O but this is likely to depend on the prevailing conditions for relevant groups of microorganisms. This study aimed to determine how climatic variables, soil abiotic conditions and variable substrate availability (leaf litter) influence CH_4 and N_2O fluxes in a semi-evergreen tropical forest on free-draining soil.**
- **CH_4 and N_2O fluxes were measured during the transition from the dry to the wet season in 2014 in a long-term litter manipulation experiment in Panama, Central America. Samples were taken from chambers strapped to individual stems of two common tree species and from soil chambers beneath the same trees.**
- **Soil CH_4 fluxes varied significantly between the dry and wet seasons, transitioning from a sink in the dry season to a minor source in the wet season. Soil N_2O fluxes were largely below detection limits during the dry season but were generally positive after the start of the wet season. By contrast, tree stems emitted CH_4 and N_2O throughout the study.**
- **There was no clear effect of litter manipulation on GHG fluxes but significant species-treatment interactions for CH_4 fluxes from stems and N_2O fluxes from stems and soil indicate complex relationships between tree species and decomposition processes that can influence GHG dynamics.**
- **Collectively, the results of this study show that tropical trees can act as conduits for trace GHGs originating from deeper soil horizons and anaerobic microsites, even when they are growing on free-draining soils. Coupled with the finding that the soils may be a weaker sink for CH_4 than previously thought, these findings could have substantial impacts on global greenhouse gas budgets. Higher stem trace GHG fluxes resulting from litter addition suggest that elevated CO_2 atmospheric concentrations could increase global soil and tree stem trace GHG emissions.**

3.1 Introduction

Methane (CH_4) and nitrous oxide (N_2O) are the second and third most important greenhouse gases (GHGs) after carbon dioxide (CO_2), with radiative effects 25 and 298 times greater than CO_2, respectively (Houghton *et al.*, 2001). Interest in GHG exchange in tropical forests has grown in recent years, particularly in saturated wetland areas of the tropics such as the Amazon (Graffman *et al.*, 2008) and mangrove swamps (Kreuzwieser *et al.*, 2003; Krithika *et al.*, 2008). Natural wetland methane emissions are the single largest methane source globally, of which tropical wetland emissions from a variety of sources (including waterlogged soils) are estimated as 177-284 Tg CH_4 yr^{-1} (IPCC, 2013). Globally, emissions of nitrous oxide from soils in natural ecosystems account for 37% of total global surface emissions (IPCC, 2007) and it has been estimated that 3.37-6.60 Tg N yr^{-1} is emitted to the atmosphere as N_2O (Zhuang *et al.*, 2012).

In addition to wetland soils, tree stems can also emit significant amounts of CH_4 in temperate (Gauci *et al.*, 2010) and tropical (Pangala *et al.*, 2013) wetland ecosystems. CH_4 and N_2O are produced in soils by methanogenic consortia of archaea and denitrifying bacteria, respectively; the gases diffuse into soil water which is absorbed through the roots. As the water is transported up the tree stem, the gases diffuse from the xylem through the stem tissue to the atmosphere via lenticels and other structures that aid gas exchange (Carmichael *et al.*, 2014). Findings extrapolated from glasshouse experiments suggest that hardwood trees could account for emissions of around 60 Tg CH_4 yr^{-1} (Rice *et al.,* 2010) and tree stem CH_4 fluxes in tropical peat forests in Indonesia accounted for 62-87% of total ecosystem CH_4 flux in a recent field study (Pangala *et al.* 2013). Mesocosm experiments with black alder trees typical of European temperate wetlands demonstrated that tree stems can also act as a pathway for N_2O emissions to the atmosphere (Rusch and Rennenberg, 1998). Wetland tree species have evolved a variety of specialist organs to aid oxygen transport to roots in anoxic soils such as aerenchyma, increased number of stem lenticels and adventitious roots which can transport soil-generated CH_4 in mangrove tree species (Purvaja *et al.*, 2004). Inter-species variations in wood specific density and stem lenticel numbers could be important controls of stem emissions, as Pangala *et al.* (2013) reported that tree stem CH_4 flux was negatively related to wood density and positively related to lenticel number. Tree stem emissions of N_2O have been observed in mesocosm studies of beech trees, a species that lacks aerenchyma and other adaptations to wet, anoxic soil conditions (Machacova *et al.*, 2013) but we do not know whether tree stems from lowland tropical forests (particularly from species without adaptations to anoxic conditions) are also capable of emitting N_2O.

Litter quantity can influence the rates of GHG emissions from forest soils as it provides the acetate used by acetoclastic methanogens and the nitrate used in denitrification (Teh *et al.*, 2008). However, litter manipulation treatments in subtropical forests in Southern China had no significant effect on soil CH_4 absorption nor N_2O production, implying that changes within the mineral soil are

more important than litter quantity (Tang *et al.*, 2006). The potential link between litter inputs and GHG emissions from the soil was explored further by a study of soil N_2O emissions from a lowland wet forest in Costa Rica, in which doubling leaf litter inputs increased rates of N_2O emissions by 43% relative to controls, with a corresponding decline of 42% in litter removal plots (Wieder *et al.*, 2011). The effects of litter manipulation on tree stem GHG emissions is presently unknown but as the above studies found that changes in mineral soil chemistry from litter were the primary driver of changes in fluxes, it is conceivable that litter manipulation could also affect stem emissions.

We are only just beginning to understand the role of tree stems as conduits of GHGs and the vast majority of research on this subject to date has been in wetlands. Although tropical forests on free-draining soils are not considered to be a major source of CH_4 and N_2O emissions, they cover a greater land area than wetland forests (Pan *et al.*, 2013). Several studies investigating greenhouse gas emissions from tree stems show that emissions decline with stem height, with the highest fluxes measured within 0.3-m of the soil surface (Rusch and Rennenberg, 1998; Gauci *et al.*, 2010; and Pangala *et al.*, 2013). As the large buttress roots common to many tropical tree species increase stem surface area, even minor GHG emissions from tree stems in tropical forests on free-draining soils could represent a major source of CH_4 and N_2O. Given that trees can be a major conduit of methane emissions in tropical peat forest and there are no field data of tree stem N_2O emissions from tropical forests, this study focuses on soil and tree stem fluxes of CH_4 and N_2O in a tropical forest on free-draining soils. Stem CH_4 fluxes have been shown to decrease with stem sampling height (Pangala *et al.*, 2013) and as the CH_4 and N_2O fluxes presented here are only sampled at the base of tree stems they may not be representative of fluxes higher up the stems.

The research presented in this chapter aimed to test the following hypotheses:

H1) Tree stems in tropical forests will emit CH_4 and N_2O when the conditions for GHG production in the soil are favourable.

H2) Fluxes of CH_4 and N_2O from the soil and tree stems will vary seasonally, mainly due to changes in temperature, rainfall and soil water content.

H3) Litter manipulation treatments will influence CH_4 and N_2O fluxes by altering substrate availability to microorganisms; hence, GHG emissions will be greater in litter addition treatments and lower in litter removal plots relative to controls.

3.2 Materials and Methods

3.2.1. Field area and sampling

The study was carried out within the Gigante Litter Manipulation Project (GLiMP; Sayer and Tanner, 2010) approximately 5 km south of Barro Colorado Island (BCI), Panama, Central America. The 15 plots were set up between 2000 and 2002; each plot measures 45-m × 45-m and the edges of the plots were trenched to a depth of 0.5-m, lined with plastic and then backfilled. Starting in January 2003, the litter is raked up and removed from five plots (L-) and added to five plots where it is spread as evenly as possible (L+); five plots were left as controls (CT). A full description of the experiment is given in Sayer *et al.* (2006) and Sayer and Tanner (2010). The mean annual temperature at the weather station on BCI is 26°C, mean annual rainfall is 2,600 mm and there is a strong dry season from mid-December to mid-April (Leigh, 1999). During the study period, maximum and minimum air temperatures were 32.4°C and 24.3°C respectively, soil temperature ranged from 24.9 – 29.2°C and soil water content (SWC) was between 14% and 40% (Fig. 3.1). The soil in the plots is characterised as a moderately acidic Oxisol (Cavelier, 1992).

Two common tree species were selected for this study: the fast-growing canopy tree *Simarouba amara* (Aubl.) and the shade-tolerant subcanopy tree *Heisteria concinna* (Standl.). Both species have relatively smooth bark and straight stems which facilitates sampling. A study of woody plants on BCI gives specific wood densities of 0.38 g cm^{-3} for *Simarouba* and 0.64 g cm^{-3} for *Heisteria* (Condit *et al.*, 2013). Trees were mapped and marked using handheld GPS. One individual per species was chosen per plot but only 13 of the 15 experimental plots contained live mature individuals of *Simarouba*; hence the present study included trees in four plots per treatment, making 12 *Heisteria* and 12 *Simarouba* trees in total.

Greenhouse gas fluxes from the soil were measured using permanently installed soil collars located 2-3 m to the north and south of each tree. The collars were made from 120-mm long sections of polyvinyl chloride (PVC) pipe (internal diameter 200 mm) which were embedded 30-mm into the soil. All collars were installed at least two weeks prior to sampling in March 2014 and an appropriate amount of litter was placed into the collars in the CT and L+ plots to achieve consistency with the surrounding forest floor. To determine CH_4 and N_2O emissions from the soil, a PVC lid with an inner seal of gas-tight neoprene foam was placed on top of the collar; a 15-ml air sample was taken by syringe via a septum in the lid immediately after closure and then again after 3, 6 and 10 minutes. Each sample was injected into pre-evacuated 12-ml borosilicate vials (Exetainer™, LabCo Ltd, High Wycombe, UK). The suction when removing the lid after sampling demonstrated the integrity of the seal on the soil chamber. Soil temperature at 0-6 cm depth was recorded adjacent to the collars using a Thermapen (ETI Ltd, Worthing, UK).

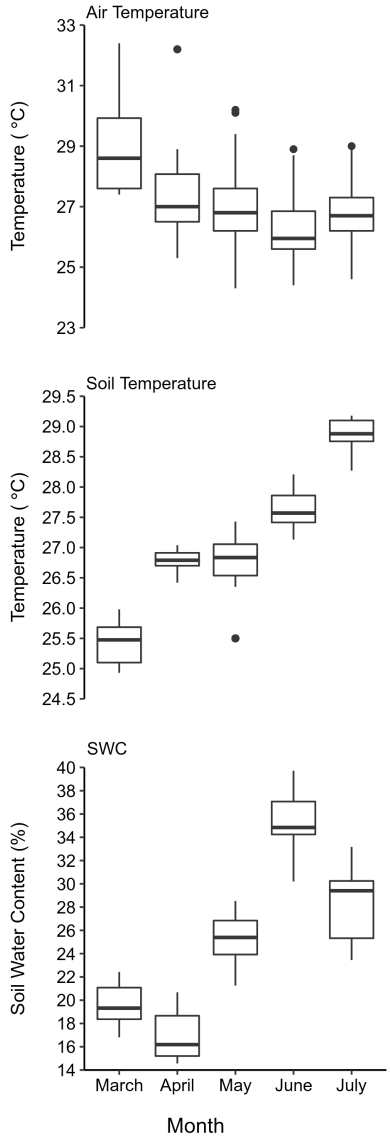

Figure 3.1. Monthly range of air temperature, soil temperature (0-10 cm depth) and soil water content (0-6 cm depth) recorded in a lowland tropical forest in Panama, Central America, from March to July 2014.

Tree stem gas fluxes were measured using a flexible chamber made from a 450-mm × 300-mm sheet of polycarbonate (Bay Plastics Ltd, North Shields, UK), lined with neoprene foam (19 mm wide, 25 mm thick; Seals+Direct Ltd, New Milton, UK). The chambers were secured to the tree stems at 0.3-m height using cam buckle straps. Gas samples were taken by syringe from a septum in the middle of the chamber at 0, 5, 10 and 15 minutes, and injected into pre-evacuated 12-ml vials as above. Air pressure and temperature outside the stem chamber were recorded at the start of sampling using a Commeter C4141 Thermometer-Hygrometer-Barometer probe (Comet Systems, Czech Republic). Collection of soil temperature and soil water content data during gas sampling was limited to 28 May - 14 June 2014 and 2 - 6 July 2014 due to equipment malfunction. Consequently, the values presented here were collected monthly from the plots as part of the long-

term monitoring of the litter manipulation experiment (Brechet *et al.* unpublished data). Soil temperature at 0-10-cm depth was measured adjacent to the collars using a soil temperature probe and volumetric soil water content at 0-6 cm depth was measured using a Thetaprobe (Delta-T Devices, Cambridge, UK) calibrated to local soil conditions following the manufacturer's instructions. Solar radiation data is provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute in 15-minute intervals, measured on a meteorological tower on BCI at 48-m height using a LiCor LI200X pyranometer (LiCor, Nebraska, USA). The daytime data were modified in R to provide a weekly mean solar radiation value using daytime measurements only.

Air samples from the tree stem and soil chambers were sampled every two weeks between 30th March 2014 and 20th July 2014. Plots were sampled in groups consisting of one plot of each treatment on consecutive days during each sampling week. Plots were sampled in the same order each week to ensure that samples were being collected at approximately the same time of day. On each day, sampling would begin around 0800 h and finish around 1400 h.

Air samples were analysed within a week of sampling to establish CH_4 concentrations. CH_4 in the samples was analysed using off-axis Integrated Cavity Output Spectroscopy (FMA-200 Fast Methane Analyser; Los Gatos Research, Mountain View, CA, USA). Air samples were returned to the Open University for analysis to determine N_2O concentrations in December 2014. N_2O in the samples was analysed using gas chromatography (Ai 94 Gas Chromatograph, Cambridge Instruments (Ellutia UK), Ely, UK).

3.2.2. Data analyses

Greenhouse gas flux data often features a small number of extreme outliers; although these values are not necessarily due to measurement error, they maybe the result of e.g. biological activity such as termites or heartwood rot (Megonigal *et al.*, 2008; Covey *et al.*, 2012) which could obscure patterns due to tree species identity or climatic variables. Consequently, the data were inspected visually and extreme outliers that lay outside of the 5th - 95th interquartile range were removed. As a result, 20 out of 201 CH_4 tree stem fluxes were removed and 2 out of 188 soil chamber CH_4 fluxes were removed. 3 of 115 tree stem N_2O fluxes were determined to be outliers and 1 of 108 flux values for soil chamber N_2O was outside the 5th-95th interquartile range. All statistical analyses were conducted with and without outliers and full results of the analyses including extreme outlier values are given in Appendix I.

All data analyses were conducted in R 3.3.2 (R Core Team, 2016) using the lme4 package for mixed effects models (Bates *et al.*, 2015). Gas fluxes were calculated for each chamber following Baird *et al.* (2010), whereby the least squares linear regression slope of the four sample

concentrations is plotted against sampling time and the slope to give the gas flux in μg m^{-2} h^{-1}. Gas flux measurements were only used for further statistical analysis if the R^2 of the regression was >0.7; this cut-off point was chosen following Alm *et al.* (2007; cited in Cooper *et al.*, 2014), who noted that low fluxes (especially those near to zero) tend to have low R^2 values.

Effects of climatic variables on soil and stem GHG fluxes were assessed using linear mixed effects models (*lmer* function) with air temperature, precipitation and their interaction as fixed effects and plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and p-values to check for model improvement (Pinheiro and Bates 2000). Having identified which climatic variables to include as covariates, tree species, litter treatment, and their interaction were added as fixed effects. These nested models were then compared as above to arrive at a new minimum adequate model. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. The final model fit was inspected using diagnostic plots. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ but due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

3.3 Results

3.3.1 Seasonal variation in CH_4 fluxes

Soil CH_4 fluxes varied significantly between the dry and wet season ($p < 0.001$, $r^2 = 0.454$, $\chi^2 = 36.4$; Fig. 3.2.a), whereby soils acted as a methane sink during the dry season and switched to being a source within 2-3 weeks of the start of the wet season. There was no clear seasonal pattern for CH_4 fluxes from tree stems; although stem CH_4 fluxes tended to be larger during the wet season, they were not significantly so (Fig. 3.2).

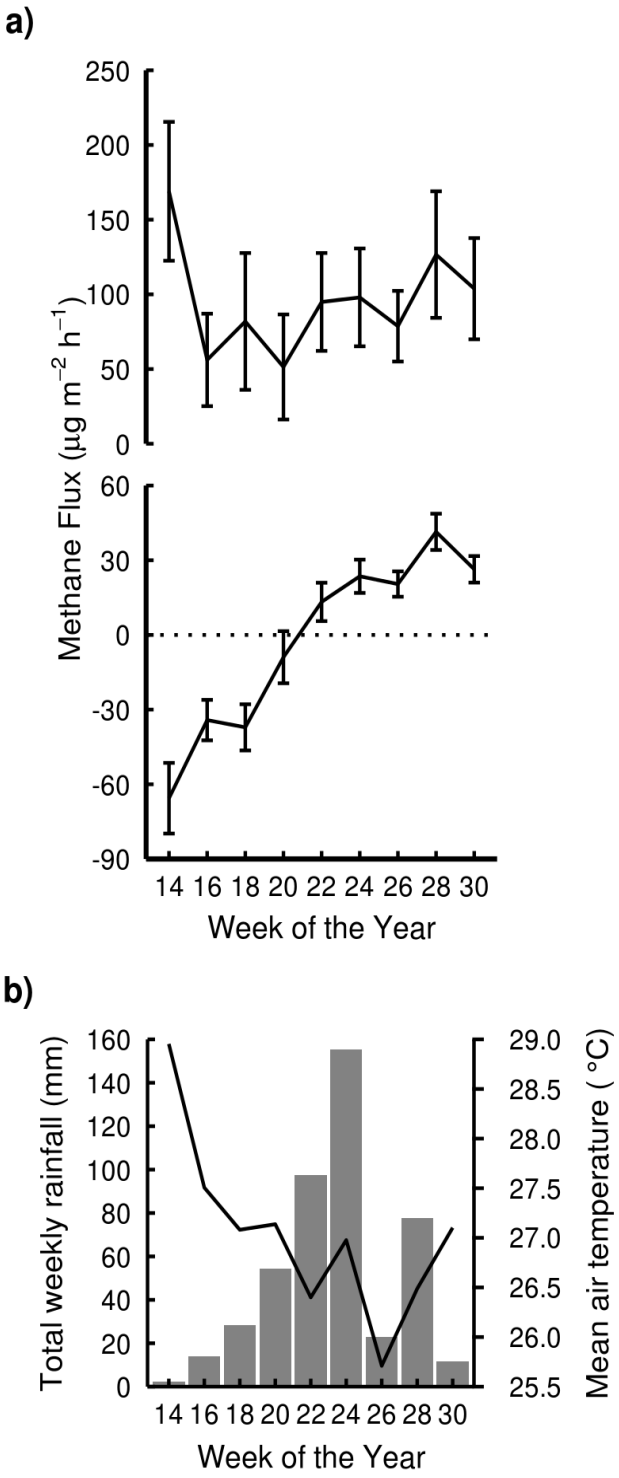

Figure 3.2 a) Seasonal patterns of methane (CH_4) fluxes from tree stems (top panel) and soil (bottom panel) in a lowland tropical forest on free-draining soil in Panama, Central America, showing the combined weekly mean stem CH_4 fluxes measured at 0.3-m height from *Heisteria concinna* and *Simarouba amara* stems and weekly mean soil CH_4 fluxes measured over chambers during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30); error bars show the standard error means for n = 4; **b)** Total rainfall in the week of sampling measured at a rainfall gauge on BCI (bars) and air temperature measured in the plots during gas sampling (line).

3.3.2. Soil chamber fluxes of CH_4

Soil CH_4 fluxes measured over chambers under individuals of *Heisteria* remained predominantly negative until week 24 of sampling, indicating dry season uptake of CH_4 before transitioning to positive fluxes (i.e. emission) four weeks after the first heavy rainfall of the year (Fig. 3.3). Soil CH_4 fluxes underneath *Simarouba* became positive two weeks after the first heavy rains in week 22. The median flux beneath *Heisteria* was 8.33 µg m^{-2} hr^{-1} which is slightly higher than the median CH_4 flux of 6.25 µg m^{-2} hr^{-1} from chambers under *Simarouba*.

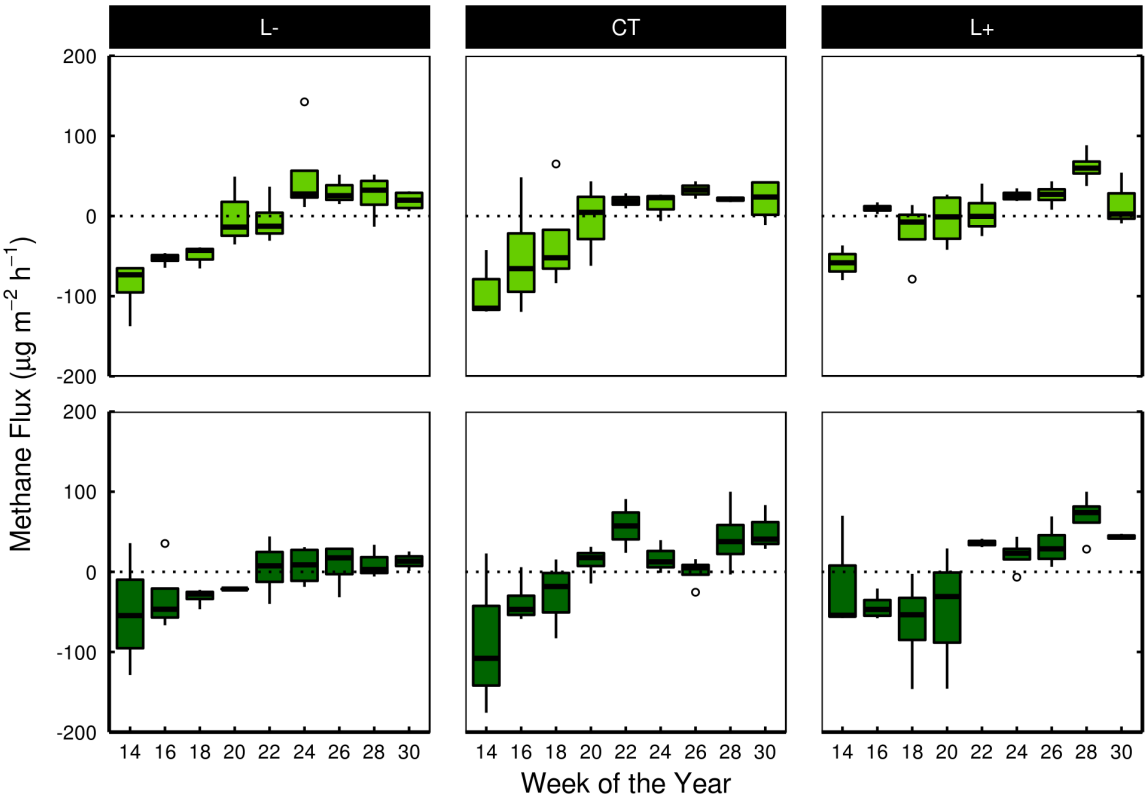

Figure 3.3 Weekly ranges of soil methane (CH_4) fluxes measured over chambers under individuals of two common tree species: Heisteria concinna (pale green boxes in the top panels) and Simarouba amara (dark green boxes in the bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30), showing the ranges (boxes and whiskers) and median lines for *n* = 4 individuals per species and treatment. Ranges are based on four replicates per species.

During the study, soil CH_4 fluxes under individuals of *Heisteria* had a greater range (-190 - 539 µg m^{-2} hr^{-1}) than under individuals of *Simarouba* (-89.4 - 450 µg m^{-2} hr^{-1}). Consequently, the mean soil CH_4 flux beneath *Heisteria* individuals was marginally more negative than that beneath *Simarouba* (-2.77 ±5.13 µg m^{-2} hr^{-1} and -2.29 ±5.46 µg m^{-2} hr^{-1} respectively). Unlike tree stem CH_4 fluxes, there were no effects of species, treatment or their interaction on soil CH_4 fluxes.

3.3.3. Tree stem fluxes of CH_4

Surprisingly, tree stem CH_4 fluxes were mostly positive throughout the study period, indicating that tropical trees emit CH_4 even when they are growing on free-draining soils. There were no significant differences in stem fluxes of CH_4 between tree species and no overall effects of litter manipulation on stem CH_4 fluxes. However there was a significant species × treatment interaction, whereby *Heisteria* stems had higher CH_4 fluxes in litter addition plots and *lower* stem fluxes in litter removal plots compared to *Simarouba* stems ($p < 0.001$, $r^2 = 0.567$, $\chi^2 = 24.5$; Fig. 3.4). Overall, the median CH_4 flux was very similar between the two species, with 72.6 μg m^{-2} hr^{-1} and 75.1 μg m^{-2} hr^{-1} for *Heisteria* and *Simarouba* respectively. Tree stem CH_4 fluxes in individuals of *Heisteria* were mostly positive, with a mean flux of 101 ±14.9 μg m^{-2} hr^{-1} over the dry-wet season transition however the mean flux for *Simarouba* was lower at 87.7 ±18.5 μg m^{-2} hr^{-1}. The median stem flux remained relatively constant throughout the study (Fig. 3.3).

Stem CH_4 fluxes in *Simarouba* displayed greater inter-week variability (Fig. 3.3) than those from *Heisteria* stems but there were many more outliers in *Heisteria* (Appendix 1). CH_4 stem fluxes from *Heisteria* ranged from -156 to 598 μg m^{-2} hr^{-1}. *Simarouba* had a greater range throughout the study, ranging from a low of -276 μg m^{-2} hr^{-1} to a maximum of 678 μg m^{-2} hr^{-1}.

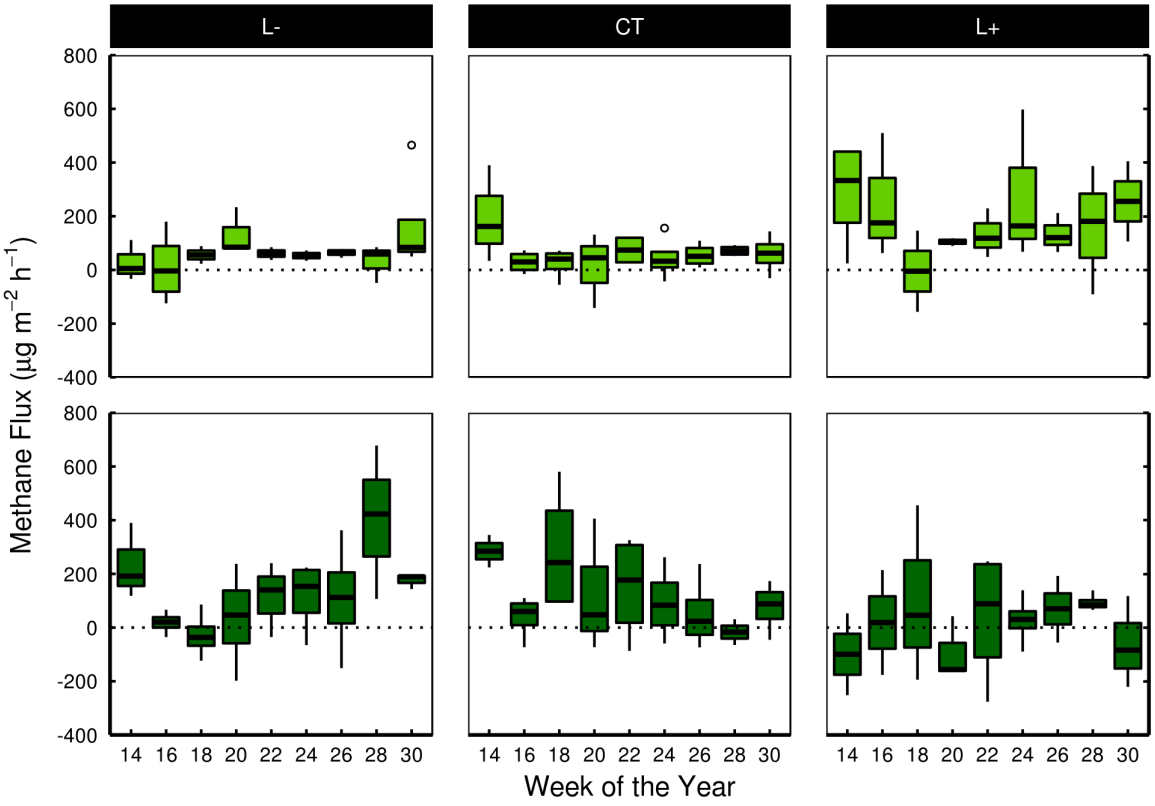

Figure 3.4 Weekly methane (CH_4) fluxes from tree stems in a lowland tropical forest in experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green boxes in top panels) and *Simarouba amara* (dark green in bottom panels), during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30), showing the ranges (boxes and whiskers) and median lines for *n* = 4 individuals per species and treatment. Ranges are based on four replicates per species.

3.3.4. Controls of soil and stem CH_4 fluxes

Soil CH_4 fluxes beneath *Heisteria* tended to increase with soil water content ($p < 0.1$, $r^2 = 0.424$, $\chi^2 = 3.4$; Fig. 3.7) but there was no significant relationship between soil CH_4 fluxes and soil temperature, air temperature (Figs. 3.5 & 3.6) or rainfall. Soil CH_4 fluxes were marginally affected by the air temperature × rainfall interaction ($p < 0.1$, $r^2 = 0.446$, $\chi^2 = 5.88$; Fig. 3.2). Tree stem fluxes had no significant relationship with soil temperature, air temperature, rainfall or solar radiation.

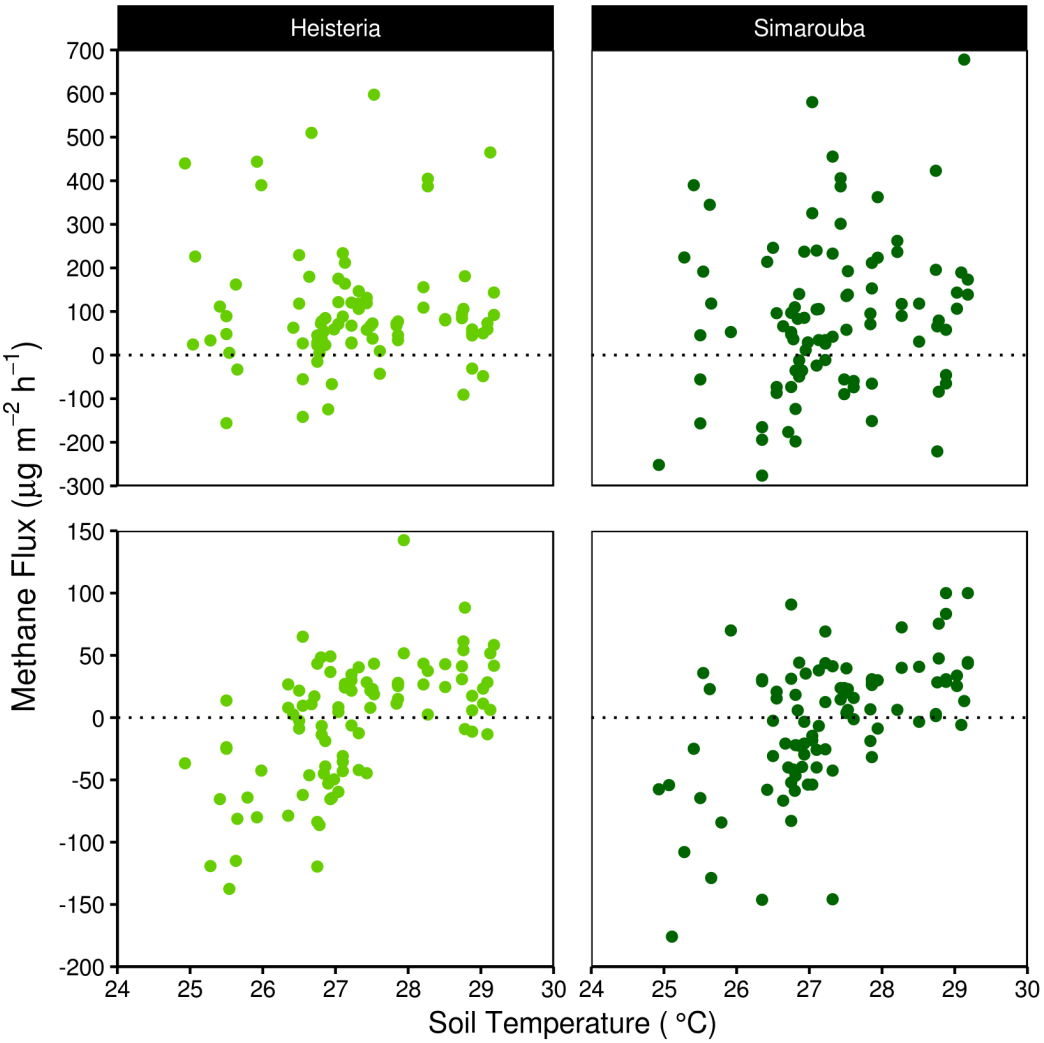

Figure 3.5 Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem CH_4 fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean CH_4 flux of two chambers beneath each tree from March to July 2014. Stem and soil CH_4 fluxes were pooled between treatments to make the above plot. Soil CH_4 fluxes indicate an apparent positive relationship with soil temperature however no significant relationship was found.

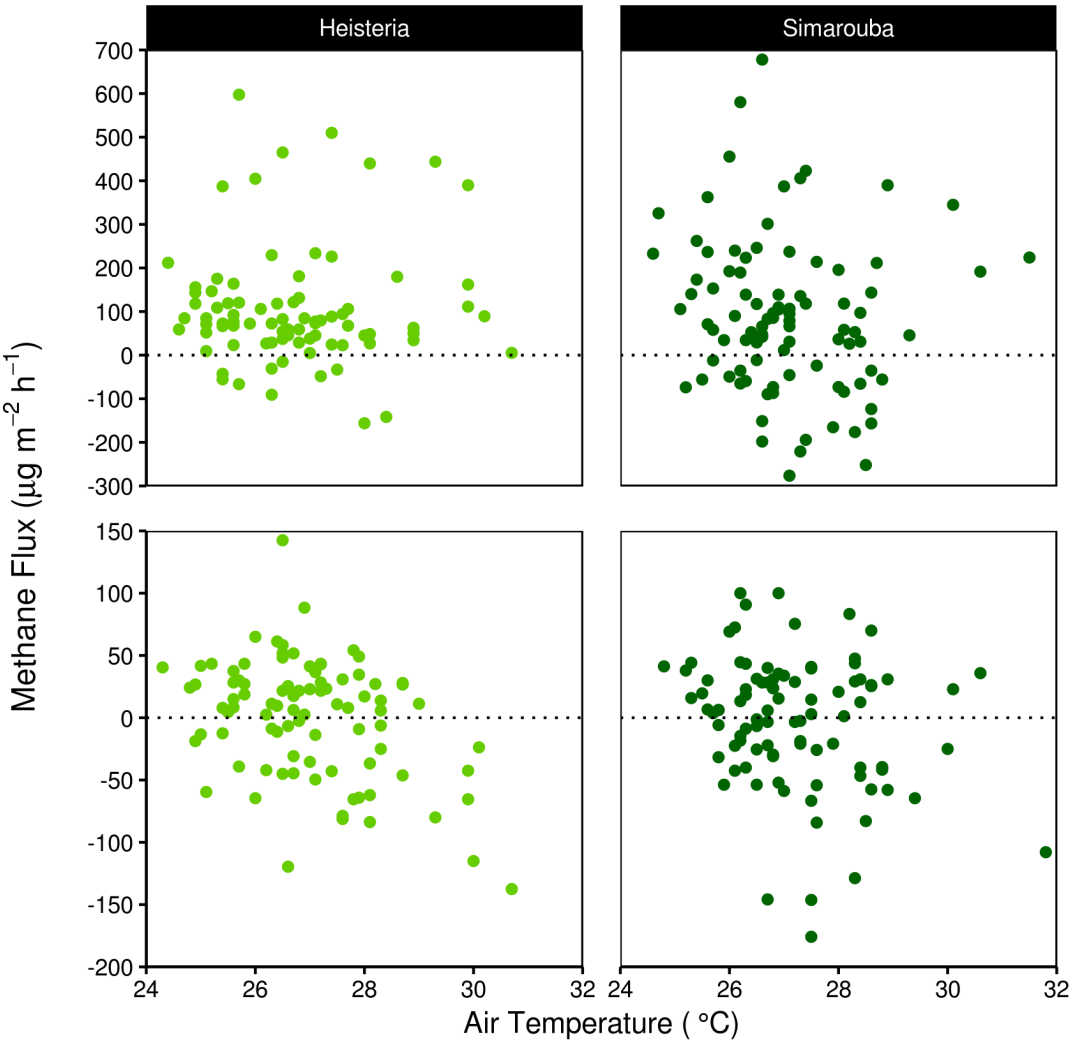

Figure 3.6 Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and air temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean flux of two chambers beneath each tree from March to July 2014. Stem and soil CH_4 fluxes were pooled between treatments to make the above plot. Air temperature was not a significant controlling variable of tree stem or soil chamber CH_4 fluxes throughout the sampling period.

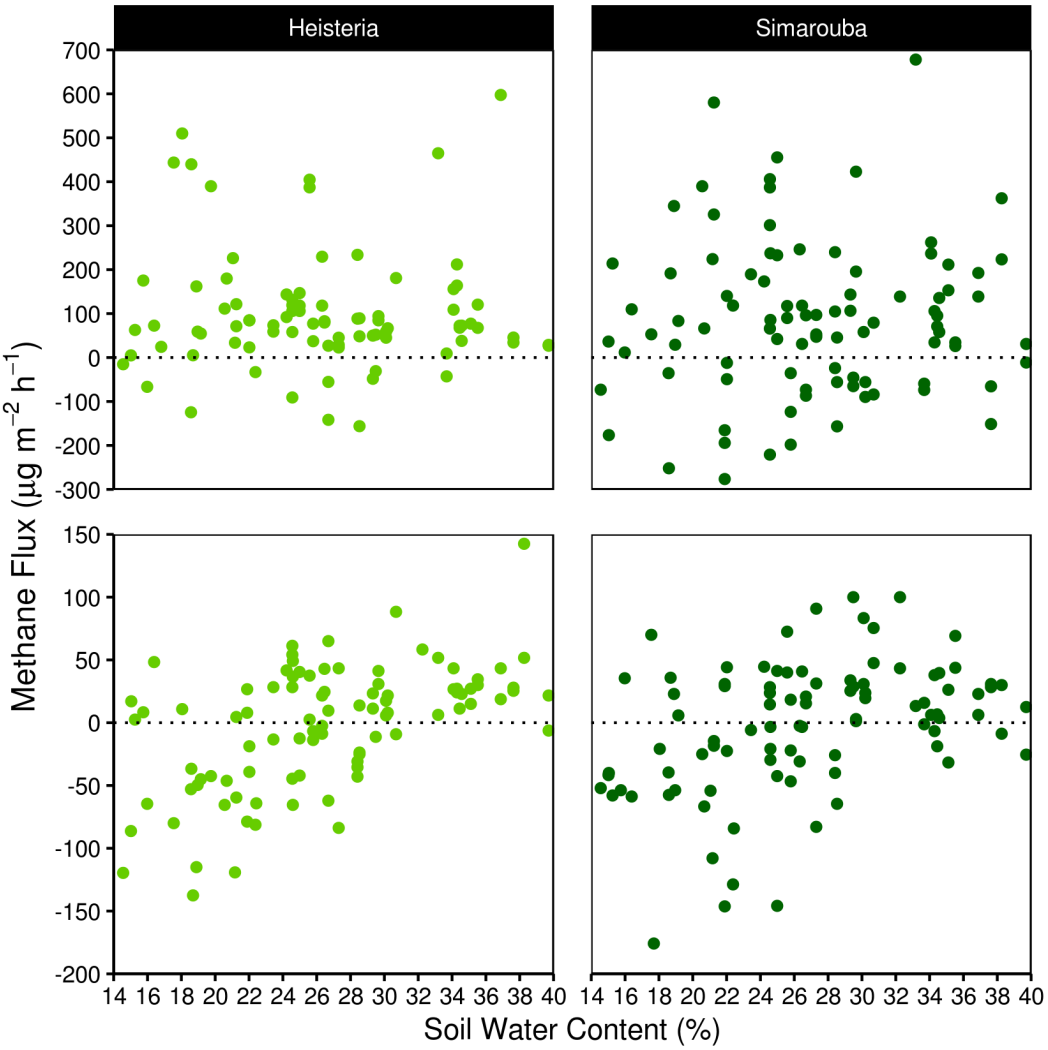

Figure 3.7 Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil water content in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean flux of two chambers beneath each tree from March to July 2014. Stem and soil CH_4 fluxes were pooled between treatments to make the above plot.

3.3.5. Seasonal variation of N_2O fluxes

There was no clear season pattern in tree stem or soil N_2O fluxes during the study period but N_2O concentrations in air samples collected from the soil collars during the dry season were mostly below the limits of detection.

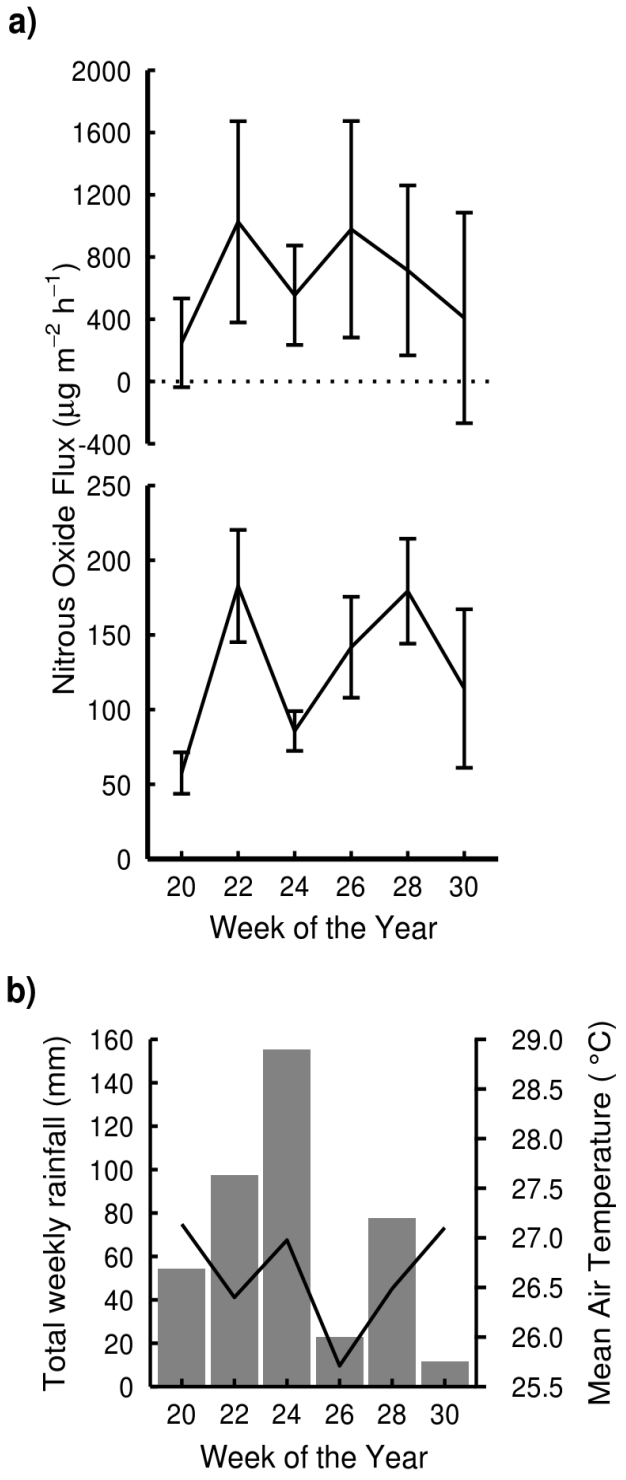

Figure 3.8 a) Seasonal patterns of nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil (bottom panel) in a lowland tropical forest on free-draining soil in Panama, Central America, showing the combined weekly mean stem CH_4 fluxes measured at 0.3-m height from *Heisteria concinna* and *Simarouba amara* stems and weekly mean soil fluxes measured over chambers during the wet season (weeks 20-30); concentrations of N_2O in dry season samples were below the limits of detection; error bars show the standard

error means for *n* = 4; **b)** Total rainfall in the week of sampling measured at a rainfall gauge on BCI (bars) and air temperature measured in the plots during gas sampling (line).

3.3.6. Soil chamber fluxes of N_2O

Median soil N_2O fluxes from chambers beneath *Heisteria* (110 µg m^{-2} h^{-1}) and *Simarouba* (87.9 µg m^{-2} h^{-1}) were lower than the median stem fluxes. Fluxes beneath *Heisteria* individuals ranged from -190 to 539 µg m^{-2} h^{-1} with fluxes under *Simarouba* trees ranging from -89.4 to 450 µg m^{-2} h^{-1}. During the wet season, mean soil N_2O fluxes were 138 ±21.7 µg m^{-2} h^{-1} beneath *Heisteria* and 114 ±15.7 µg m^{-2} h^{-1} beneath *Simarouba*. There was a marginally significant species x litter treatment interaction for soil N_2O fluxes ($p < 0.1$, $r^2 = 0.137$, $\chi^2 = 6.1$), whereby soil N_2O fluxes measured beneath *Heisteria* individuals were greater than fluxes measured under *Simarouba* in litter addition plots only (Fig. 3.9).

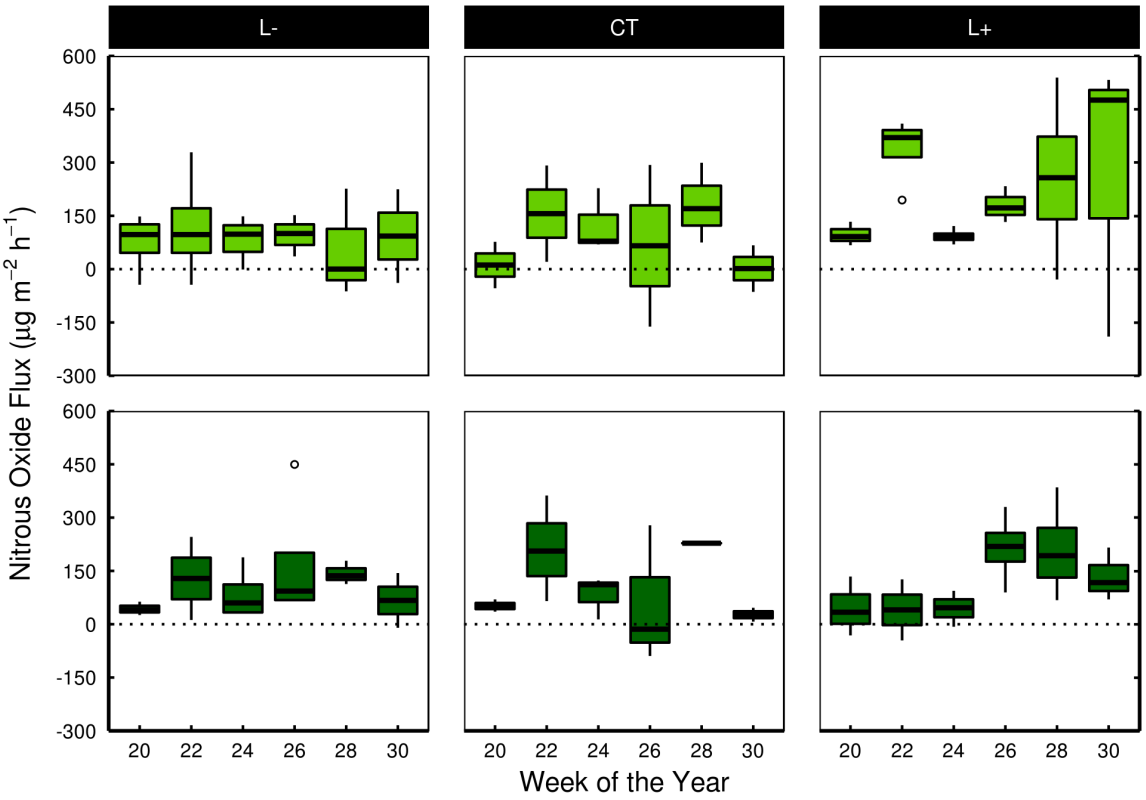

Figure 3.9 Weekly ranges of soil N_2O fluxes measured over chambers under individuals of two common tree species *Heisteria concinna* (light green in top panels) and *Simarouba amara* (dark green in bottom panels) in a lowland tropical forest with experimental litter manipulation (litter removal (L-), control (CT) and litter addition (L+)) on free-draining soil in Panama, Central America, during the wet season. Dry season data is not present as N_2O concentrations in dry season air samples were below the limits of detection. Ranges are based on four replicates per species.

3.3.7. Tree stem fluxes of N_2O

Although there were no significant differences in stem N_2O fluxes between species, median N_2O fluxes from *Heisteria* were much lower than those from *Simarouba* (101 µg m^{-2} h^{-1} and 1001 µg m^{-2} h^{-1}, respectively) over the course of the study. N_2O fluxes from *Heisteria* stems were less variable (range: -2857 to 4270 µg m^{-2} h^{-1}) compared to *Simarouba* (range: -3770 to 8361 µg m^{-2} h^{1}). Overall, a greater proportion of stem fluxes in *Simarouba* were positive and the mean stem flux from *Heisteria* was 80 ±234 µg m^{-2} h^{-1} compared to 1193 ±361 µg m^{-2} h^{-1} for *Simarouba* (Fig. 3.10). There was a significant species x litter treatment interaction for stem N_2O fluxes ($p < 0.05$, $r^2 = 0.089$, $\chi^2 = 9.6$), whereby fluxes from *Simarouba* stems were greater than those from *Heisteria* in litter addition and control plots, but not in litter removal treatments (Fig. 3.9).

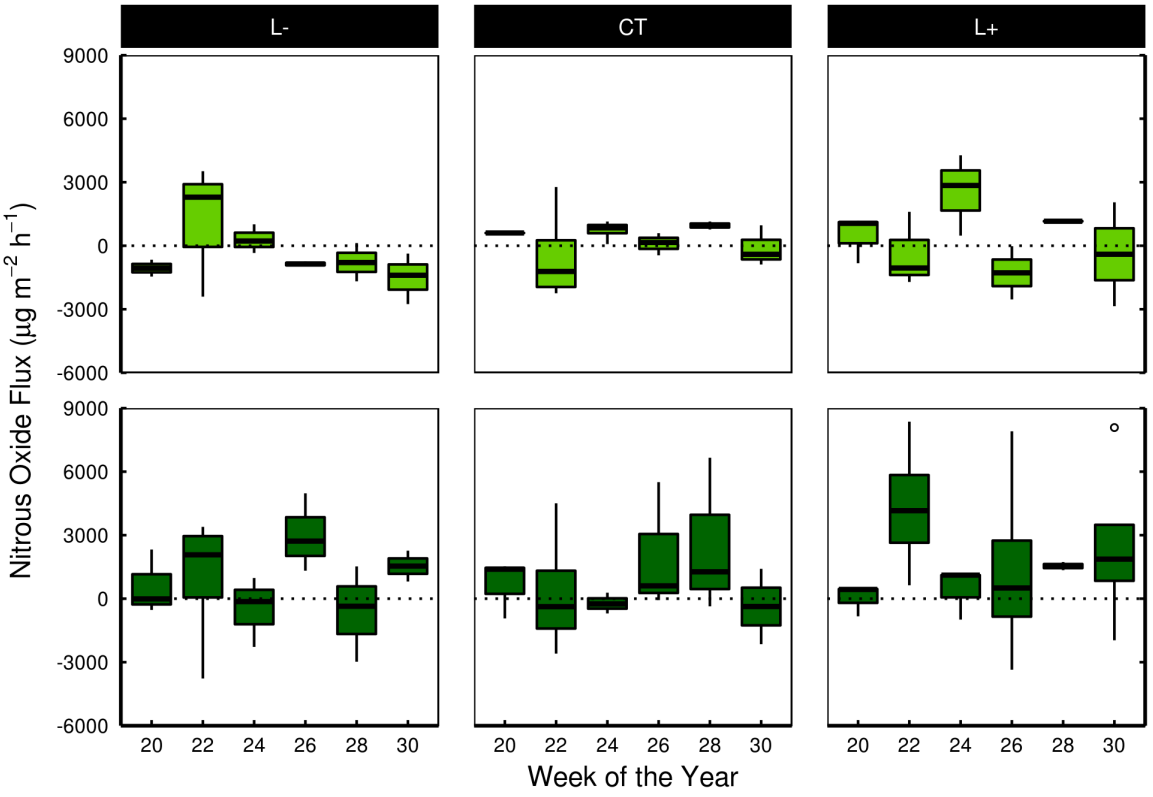

Figure 3.10 Box and whisker plot of N_2O fluxes from tree stems in a lowland tropical forest with experimental litter manipulation (litter removal (L-), control (CT) and litter addition (L+)) on free-draining soil in Panama, Central America, showing stem fluxes in two common tree species *Heisteria concinna* (light green in top panels) and *Simarouba amara* (dark green in bottom panels) measured at 0.30-m height during the wet season. Dry season data is not shown as N_2O concentrations in dry season air samples were below the limits of detection. Ranges are based on four replicates per species.

3.3.8. Controls of tree stem and soil N_2O

Similar to CH_4 fluxes, there was no clear relationship between tree stem or soil N_2O fluxes and soil temperature (Fig. 3.11) or air temperature (Fig. 3.12). In addition, tree stem fluxes were not significantly related to weekly mean solar radiation. However, soil N_2O fluxes increased significantly with soil water content ($p < 0.05$, $r^2 = 0.169$, $\chi^2 = 12.5$; Fig. 3.13).

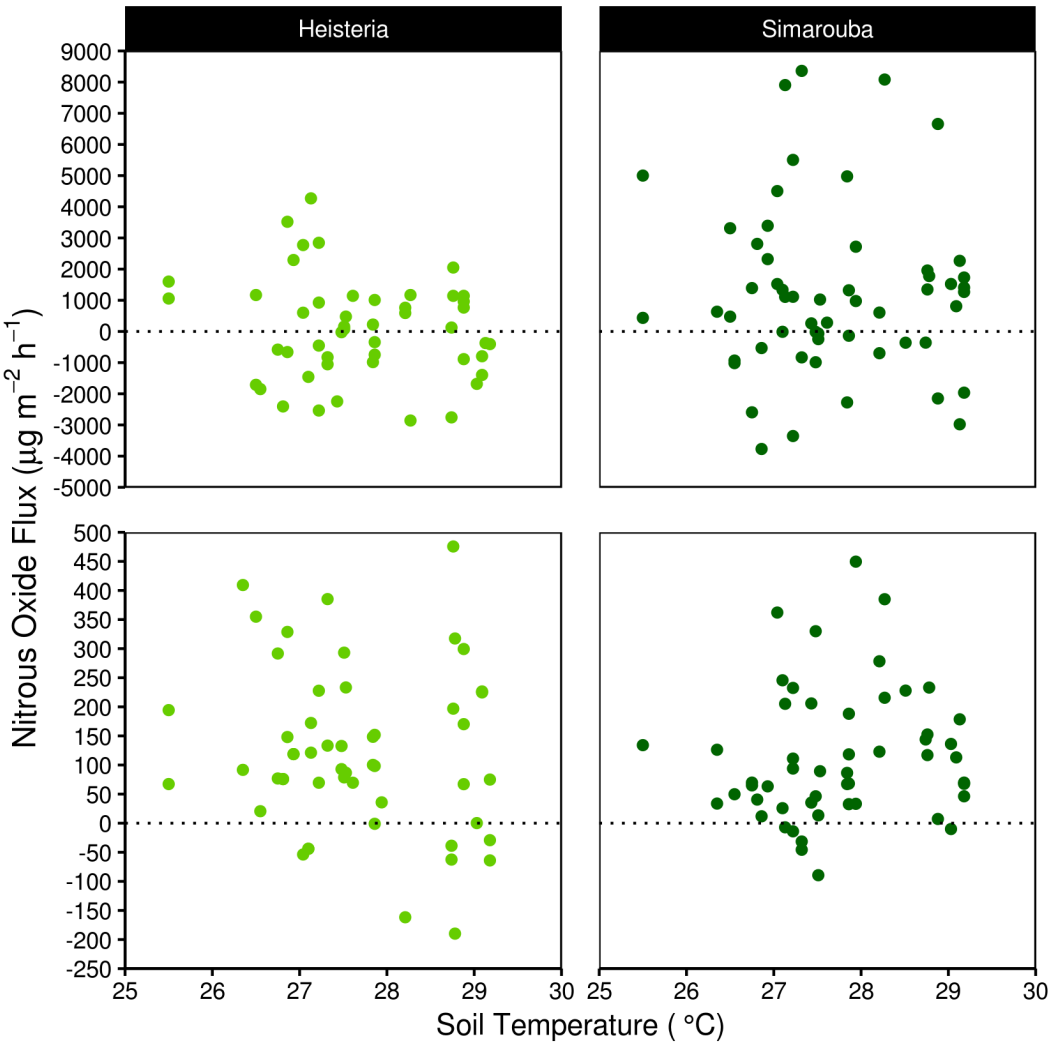

Figure 3.11 Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean flux of two chambers beneath each tree from May to July 2014. Stem and soil N_2O fluxes were pooled between treatments to make the above plot.

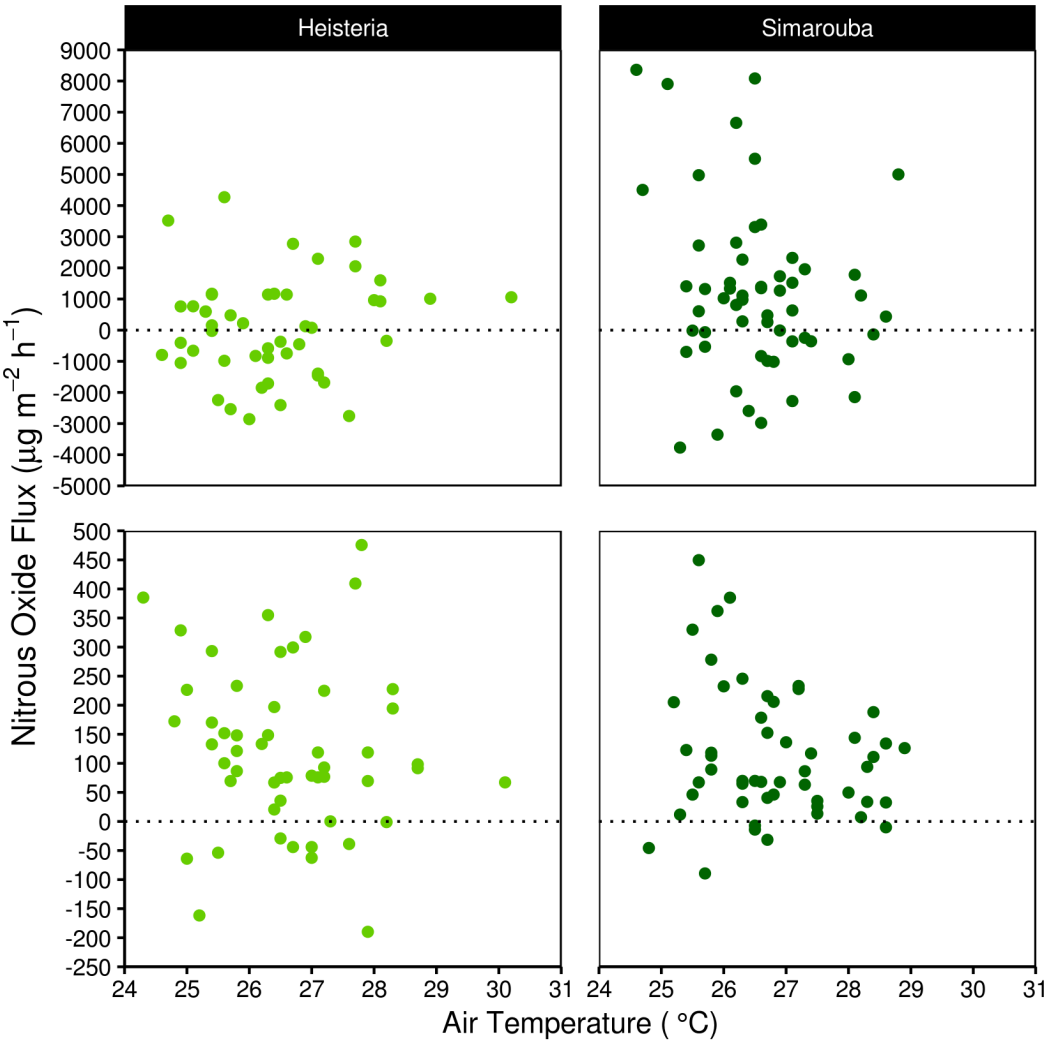

Figure 3.12 Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and air temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean flux of two chambers beneath each tree from May to July 2014. Stem and soil N_2O fluxes were pooled between treatments to make the above plot.

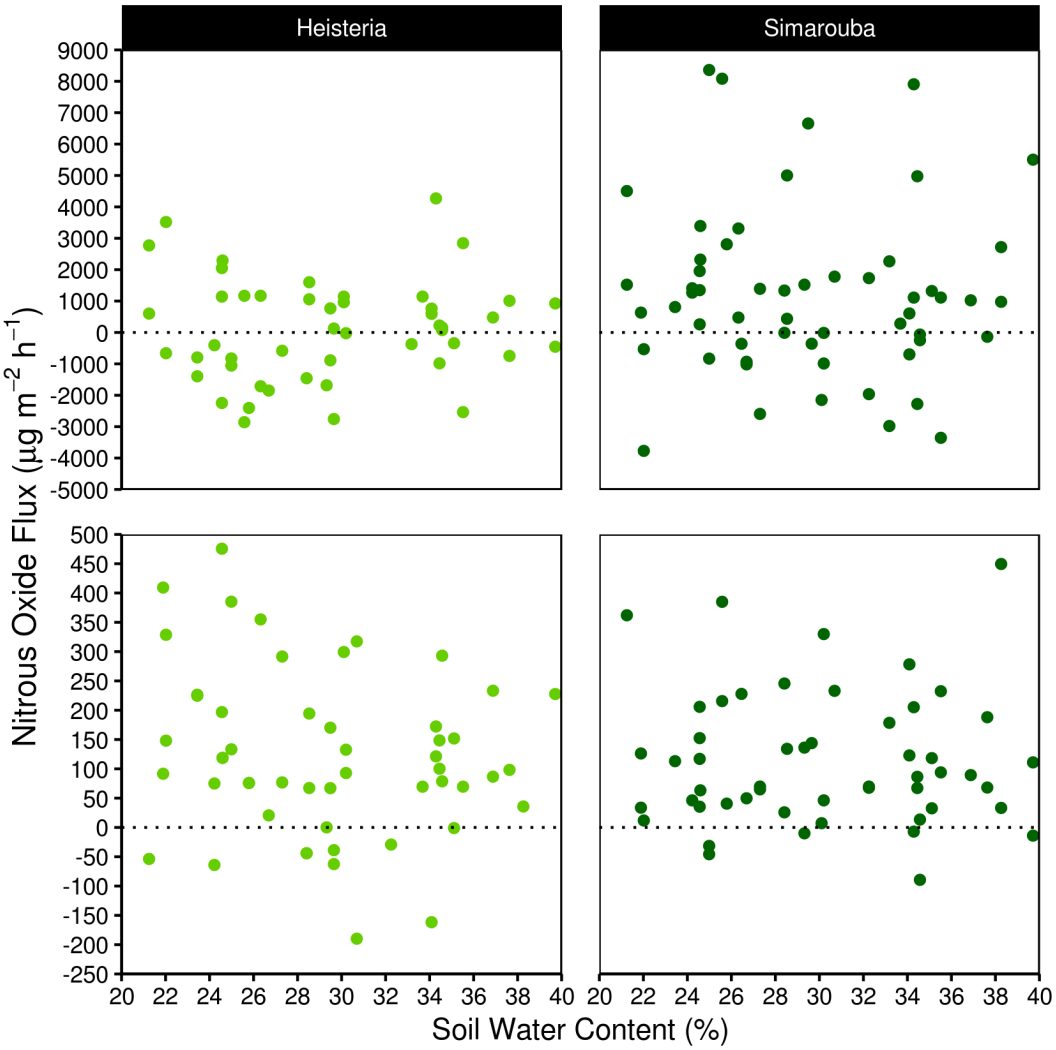

Figure 3.13 Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil water content in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green) and *Simarouba amara* (dark green) and the mean flux of two chambers beneath each tree from May to July 2014. Stem and soil N_2O fluxes were pooled between treatments to make the above plot.

3.4. Discussion

Soil trace GHG fluxes were strongly seasonal in this lowland tropical forest in Panama, whereby the soil acted as a CH_4 sink during the dry season, when shallow portions of the soil profile are well aerated and methanogenic archaea are probably dormant. With the onset of the wet season at the beginning of May (Fig. 3.2), conditions became more favourable for methanogenesis and the soil CH_4 fluxes shifted from strongly negative towards positive values. Although the seasonal pattern was less pronounced for soil N_2O fluxes, 98% of samples collected from tree stem and soil chambers during the dry season had concentrations of N_2O below detection limits.

3.4.1 Abiotic controls of GHG fluxes

Surprisingly, tree stem CH_4 and N_2O fluxes did not respond to changes in SWC (Figs. 3.7 and 3.13) and there was no relationship between CH_4 emissions or uptake from soils and SWC but N_2O emission was enhanced by SWC. Other studies of tropical and subtropical swamp forest and rainforest sites show a clear linear positive relationship between SWC and CH_4 flux (Yu *et al.*, 2008; Rowlings *et al.*, 2012 and Hall *et al.*, 2013). However CH_4 emissions from tropical rainforest soils can also vary depending on water table depth and methane oxidation can still occur within water-filled pore spaces when the water table was closer to the surface (Mitsch *et al.* 2010), indicating that CH_4 emissions from soils are episodic and highly dependent on SWC at different depths. In the present study, measurements of SWC were made from 0-6 cm depth. However, previous work demonstrated higher CH_4 emissions at 0.15 - 0.3 m depth in free-draining soils (Mitsch *et al.* 2010) and surface SWC may therefore not be relevant for CH_4 fluxes when soils are not waterlogged. A rainfall manipulation experiment in the Luquillo Experimental Forest in Puerto Rico showed that even under simulated high precipitation rates, aerobic (e.g. methanotrophy) and anaerobic (e.g. methanogenesis) processes can occur simultaneously in upland tropical soils (Hall *et al.*, 2013).

Similarly, N_2O emissions usually increase with SWC as the anaerobic (and more productive) process of denitrification becomes dominant. Nonetheless, there was no significant relationship between soil water content and tree stem N_2O emissions in this study. This discrepancy could be explained by the timing and frequency of measurements, as mesocosm studies have demonstrated that N_2O emissions were greatest 24 hours (Machacova *et al.* 2012) or ~45 hours (Liengaard *et al.* 2014) after rewetting but then declined rapidly. It is therefore probable that sampling times did not always coincide with maximum denitrification rates.

The lack of clear climatic and seasonal responses in tree stem fluxes could be attributed to variation in water availability and GHG production with soil depth. Soil pit studies performed at the study site from May 2006 to February 2009 found that soil moisture increased significantly

with depth, whereas CH_4 concentrations decreased with depth to *c.* 1.25-m, and N_2O concentrations increased with depth (Koehler *et al.*, 2009a). Accordingly, we would expect higher CH_4 fluxes and lower N_2O fluxes from stems when trees source water from shallower soils and the reverse when trees access water from deeper soil horizons. Interestingly, tree stems also acted as a sink for GHG gases in this study. It is possible that changes in CH_4 and N_2O concentrations in soil water at different depths could generate a diffusion gradient from the atmosphere into tree stems, thus resulting in tree stem uptake of GHGs, possibly as a result of active consumption by epiphytic and endophytic methanotrophs.

Although a number of studies have shown that rates of soil methanotrophy increase with soil temperature (Steinkamp *et al.*, 2001; Butterbach-Bahl *et al.*, 2002 and Yvon-Durocher *et al.*, 2014), a comparison of temperate and tropical forested ecosystems observed only a weak temperature response of CH_4 uptake at the tropical rainforest site (Luo *et al.* 2013). The lack of a significant temperature effect on soil or tree stem CH_4 (Figs 3.5 & 3.6) and N_2O fluxes (Figs 3.11 & 3.12) in this study is not surprising, as soil temperature was consistently >24ºC and the largest difference in soil temperature between March and August 2014 was <6ºC. Temperature differences have a more profound effect on GHG fluxes when temperatures drop below 15 ^{o}C because at higher temperatures, other factors such as diffusion rate (affected by soil density etc; Le Mer and Roger, 2001; Liu *et al.*, 2007) and drought effects (that have broader systemic impacts; Davidson *et al.*, 2008; Itoh *et al.*, 2010) become relatively more important.

It is worth noting that there was an El Niño event in 2014 (Menkes *et al.*, 2015) that lead to a strong dry season in Panama, with higher temperatures and lower rainfall than average. Previous El Niño events have reduced CH_4 emissions and stabilised atmospheric concentrations, with a mean reduction in global emissions of -9 Tg CH_4 yr^{-1} (Hodson *et al.*, 2011). In the present study, seasonal effects may therefore have been exaggerated by the more severe dry season compared with other years.

Recent research has shown that nitrification involves both bacteria and archaea. Ammonia oxidizing bacteria (AOB) and ammonia oxidising archaea (AOA) both create N_2O, however N_2O production in AOA is lower than in AOB, as they lack the additional reductase enzymes present in AOB. In turn, this leads to a lower yield of N_2O versus nitrite in AOA, which is only 50% of that in AOB (Prosser and Nicol, 2008; Hink *et al.*, 2016). However, soil pH can affect the activity of each group of ammonia oxidisers, whereby low pH conditions favour AOA; in regions with pronounced dry seasons, this could lead to lower N_2O fluxes from soil surfaces. The soils in the study plots were moderately acidic, which would favour AOA (Hink *et al.*, 2016); when coupled with the effects of the dry season, exacerbated by the 2014 El Niño event, the predominance of AOA could explain why dry season N_2O fluxes were so low.

Soil N_2O fluxes mirrored those of CH_4 in showing significant seasonality that coincided with increasing rainfall (and soil water content) from the onset of the wet season at the beginning of May through to the end of sampling in late July (Fig. 3.8). Similar seasonal patterns of CH_4 and N_2O are expected because the dynamics of both GHGs are linked to soil water content and decomposition processes. Decomposition plays a role in methanogenesis because litter is the primary source of acetate (McInerney *et al.*, 2009) and competition for acetate between methanogenic archaea and Fe(III)-reducing bacteria in rainforest soils is an important factor controlling CH_4 (Teh *et al.*, 2008) Similarly, inorganic nitrogen is released from litter during decomposition and nitrous oxide emissions are positively correlated with nitrate concentrations (Koehler *et al.*, 2009b). Decomposition rates increase two-fold in the wet season compared to the dry season (Wieder and Wright, 1995; Kiese *et al.*, 2003) and nitrate availability increases with enhanced decomposition after wetting, leading to spikes in N_2O fluxes that coincide with rainfall (e.g. weeks 22 and 28 in Fig. 3.8.). This could also partly explain why N_2O was below the limits of detection in almost all dry season samples from soil chambers. Hence, in free-draining tropical forest soils, the combined spatial variability in moisture, oxygen concentrations, decomposition processes and electron acceptors may represent a greater control on trace greenhouse gas uptake or emission than any single factor.

3.4.2 Effects of litter manipulation

Given the influence of decomposition processes on GHG production, we would expect both CH_4 and N_2O fluxes to be higher in litter addition plots as a result of greater availability in acetate and nitrate. Although there was a significant effect of litter manipulation on CH_4 fluxes from tree stems in this study (Fig. 3.4), there was not a significant effect on CH_4 fluxes from soils. It is conceivable that the low SWC during the dry season reduced potential effects of the litter manipulation by slowing decomposition rates. A study of decomposition rates in a temperate upland forest found that litter decomposition contributions to ecosystem respiration rose from 6% during a transient drought period to 37% immediately after wetting (Cisneros-Dozal *et al.*, 2007). Accelerated decomposition of leaf litter after wetting would increase the availability of acetate that is used by methanogenic consortia which would lead to increasing CH_4 fluxes during the wet season.

With a maximum SWC of ~40% in the upper 6-cm of soils within the plots (Fig. 3.1), it is likely that rates of methanotrophy at the surface were high which would mask litter manipulation effects on soil surface CH_4 emissions. Furthermore, trees take up water (and therefore CH_4) from deeper in the soil profile which would also explain why stem and soil CH_4 fluxes were unrelated, even though soil CH_4 fluxes were measured directly beneath the trees. The differences between species could well be related to rooting depth and this possibility merits further study.

Tree species and litter manipulation interacted to influence N_2O fluxes from tree stems (Fig. 3.10) and N_2O fluxes from the soil to a lesser extent (Fig. 3.9). The similarity of patterns in tree stem and soil N_2O and CH_4 fluxes strengthens the argument that although comparatively dry, aerated upper soils may retard trace GHG emissions from the soil surface, tree stem GHG fluxes originate from deeper soil layers where N_2O concentrations are higher (Koehler *et al.*, 2009a) and emissions are less impeded in transit to the atmosphere. Increased soil N_2O fluxes in small-scale litter addition plots in lowland tropical forest in Costa Rica were attributed to higher rates of dissimilatory nitrate reduction in response to the higher concentration of dissolved organic carbon which resulted in faster cycling of nitrate (Wieder *et al.*, 2011). However, hotspots and 'hot moments' often account for a large proportion of denitrification activity, such that increased litter inputs are only likely to result in increased rates of denitrification if there are sufficiently large communities of denitrifying bacteria and the soils can respond quickly to wetting events (McClain *et al.* 2003). With such high spatial and temporal variability in N_2O production, tree stem fluxes will vary widely depending on the presence of such hotspots within their rooting zones and whether the timing of sampling coincides with a hot moment of denitrification which explains the high inter-week variation in the present study (Fig. 3.10).

Thus, although there were no consistent effects of the litter treatments on GHG emissions, higher nitrous oxide emissions in litter addition plots towards the end of the study period (weeks 24-30; Fig. 3.9 & 3.10) along with an accompanying litter addition effect on CH_4 emissions (Fig. 3.3 & 3.4) reveal that there is some connection between litter quantity, substrate supply and SWC. It is probable that a more pronounced impact of litter manipulation on both soil and stem fluxes would be observed at the end of the wet season in November and December.

3.4.3 Tree stems as sources of GHG

The majority of stem fluxes of both GHGs were positive throughout the study (Figs. 3.4 & 3.10) and, unlike soil GHG fluxes, did not change significantly between seasons. However, as the majority of CH_4 and N_2O emitted via the plant pathway originates from soil methanogenic consortia and denitrifying communities, it is likely that the transport of trace GHGs through tree stems in the present study bypassed the oxygenated top soils where the majority of CH_4 oxidation (Teh *et al.*, 2005; Wolf *et al.*, 2012) and more complete denitrification (Koehler *et al.*, 2009a; Wieder *et al.*, 2011) occurs. Stem emissions CH_4 and N_2O stem fluxes reflected the composition of soil trace GHG concentrations in mesocosm studies of temperate tree saplings and herbaceous plant species (Rusch and Rennenberg, 1998; Wang *et al.*, 2008; Nisbet *et al.*, 2009), a finding that has been confirmed *in situ* for mature tree stems in a Japanese temperate floodplain forest (Terazawa *et al.*, 2007), Indian mangrove swamp (Purvaja *et al.*, 2004) and Indonesian tropical peat forest (Pangala *et al.,* 2013).

This study only measured stem fluxes from two species that were common to all the plots, however there are dozens of different tree species within the plots, with stem fluxes from each species potentially varying due to physiological traits. CH_4 and N_2O concentrations within soil pore gas and water are influenced by the diversity of tree species in the ecosystem. Comparisons between monodominant plantation forest with mature rainforest and abandoned pasture sites in Costa Rican Caribbean lowland forests found significant inter-species differences in N_2O fluxes, with the highest fluxes under late-successional species and the lowest fluxes under fast-growing early successional species (Weintraub *et al.*, 2014). Mesocosm studies of two temperate tree species common to forests on free-draining soils found that their rhizosphere could increase CH_4 uptake and decrease N_2O emissions from soils because soil nitrate concentrations were reduced under the fast-growing species which is known to affect methanogenesis and denitrification. Fast-growing species also created channels of greater gas diffusivity through higher fine root density and greater maximum root depth (Fender *et al.*, 2013). In the present study, both CH_4 and N_2O fluxes were generally higher from stems of the fast-growing pioneer *Simarouba* (Fig. 3.4 & 3.10), apart from in litter addition plots, where stem CH_4 fluxes were greater from the slow-growing shade-tolerant tree *Heisteria*. No relationships were found between solar radiation and trace GHG fluxes but larger stem fluxes in *Simarouba* suggest that canopy species are likely to have higher fluxes because they are more exposed to sunlight and have greater rates of evapotranspiration compared to subcanopy species. Higher rates of net primary productivity in canopy species, increase the release of root exudates into soil which could stimulate CH_4 and N_2O production (Topp and Pattey, 1997; Butterbach-Bahl *et al.*, 2013). Should evapotranspiration be the dominant control of tree stem CH_4 and N_2O, high evapotranspiration rates could lead to higher trace GHG fluxes however this would also lower SWC around the tree roots and therefore over time decrease CH_4 and N_2O fluxes.

The majority of outlier CH_4 and N_2O fluxes were measured from tree stem chambers (Appendix I). These fluxes (which were an order of magnitude or greater than fluxes from other tree stems of the same species and treatment type) could arise from rooting systems tapping into soil "hotspots" with larger populations of methanogenic and denitrifying bacteria than surrounding soil areas. For tree stem CH_4 fluxes, the outliers were mostly observed in *Heisteria* stems, and they increased to the maximum value over a six-week period, before returning to typical values two weeks later (Fig. 3, Appendix I). The largest N_2O flux (15,657 μg m^{-2} h^{-1}) was measured from an individual *Heisteria* stem in a litter addition plot as was the second-highest CH_4 flux (13358 μg m^{-2} h^{-1}). However, the largest flux of CH_4 was measured from a *Heisteria* stem in a litter removal plot (19,350 μg m^{-2} h^{-1}), which suggests that something other than substrate availability must be driving these extremely high GHG fluxes. These extreme outliers were removed prior to analysis, as they masked broader trends in the data. Nevertheless, they are still pertinent to understanding trace GHG gas exchange in lowland tropical rainforests, as they can influence estimates of ecosystem scale

CH_4 and N_2O fluxes, especially if they are relatively common occurrences. Further, there may be predictable relationships between such high GHG fluxes and environmental variables, which would only become apparent with more in-depth, longer-term study.

In addition to abiotic and tree species effects on fluxes of trace GHGs, it is possible that other biological activity could influence CH_4 and N_2O fluxes and explain the few extreme outliers measured in this study (Appendix I). Measurement error aside, there are two possible biological explanations for unusually high GHG fluxes in lowland tropical forests: 1) Termite activity is estimated to account for 11 Tg CH_4 y^{-1} (Kirschke *et al.*, 2013) as it is possible that a termite nest within the soil or a tree stem could elevate stem CH_4 fluxes in excess of what is otherwise typical for this ecosystem; 2) Heartwood rot is not fully understood but may be more prevalent than we think and laboratory analyses of tree cores extracted from mature tree stems in temperate upland forests in the USA and China found that heartwood rot may increase GHG fluxes from tree stems (Covey *et al.*, 2012; Wang *et al.*, 2016).

3.5 Conclusions

This is the first study of trace GHG fluxes from tree stems in a tropical forest on free-draining soils, as well as the first *in situ* study of N_2O fluxes from tree stems in a tropical ecosystem. These results suggest that tropical tree stems on free-draining soil may represent a hitherto unaccounted for conduit of GHG emissions from deeper soil horizons. Litter addition had significant effect on stem CH_4 and N_2O fluxes which, in an increasingly CO_2-rich atmosphere could have ramifications globally on terrestrial CH_4 and N_2O emissions.

The results presented here demonstrate that the soil CH_4 sink decreased during the transition from the dry to the wet season at which point the soil became a small source of CH_4. Importantly, tree stems consistently produced positive fluxes of CH_4 during the wet and dry seasons, which potentially offsets some or all of the dry season soil sink. Tree stem fluxes of N_2O were only detectable in the wet season and were also consistently positive. Given that tropical forests on free-draining soils cover a larger area than tropical wetland forests, the contribution of stem CH_4 and N_2O fluxes to ecosystems and global trace GHG budgets merits further study.

Chapter 4: Environmental controls of CH_4 and N_2O fluxes in a temperate forest on free-draining soil

Abstract

- **Tree stems on temperate upland soils can emit CH_4 but the majority of published studies describing tree stem fluxes of methane (CH_4) have focused on wetland ecosystems and only a handful of mesocosm studies have reported nitrous oxide (N_2O) fluxes from trees.**
- **Temperate forests on free-draining soils are assumed to be a CH_4 sink and a weak source of N_2O but this is likely to depend on the prevailing conditions for relevant groups of microorganisms. This study aimed to determine how climatic variables, soil abiotic conditions and tree species influence CH_4 and N_2O fluxes in a temperate woodland on free-draining soil.**
- **CH_4 and N_2O fluxes were measured monthly between February 2015 and January 2016 in Wytham Woods, Oxfordshire, UK. Air samples were taken from chambers strapped to individual stems of two common tree species and from soil chambers in the plots. To assess the influence of stem sampling height on CH_4 fluxes, samples were collected at 4 points between 0 and 2-m above the forest floor for each tree stem.**
- **Tree stem CH_4 fluxes varied significantly throughout the year and differed with sampling height, from emission at stem bases to uptake 1-m above the forest floor. Soil CH_4 fluxes transitioned from a source in early spring, to a sink during summer and then back to a source in winter. Tree stem and soil N_2O fluxes were highly variable year-round with no seasonal trends.**
- **There were no significant species differences in stem CH_4 fluxes at multiple sampling heights. Changes in air temperature, soil temperature and soil water content did not affect stem trace GHG fluxes but solar radiation was positively correlated with stem CH_4 flux.**
- **Collectively, these results show that temperate trees on free-draining soils can act as sinks of CH_4 and N_2O. As free-draining, aerated soils are the largest terrestrial CH_4 sink, this may potentially double the size of the global CH_4 sink as presently tree stem uptake is not accounted for in greenhouse gas budgets.**

4.1 Introduction

Globally, hardwood forests are estimated to be a potential annual source of ~60 Tg methane (CH_4; Rice *et al.,* 2010). Free-draining forest soils can be sinks of CH_4 and weaker sources of nitrous oxide (N_2O) under a variety of climatic conditions (Dong *et al.*, 1998; Borken *et al.*, 2003 & Fender *et al.*, 2013). A study of over 50 soil types from across Europe found that the optimum water-filled pore space for soil N_2O emissions was 70-80%, however free-draining forest soils will only rarely attain that optimum (Butterbach-Bahl *et al.*, 2013). Studies of the effects of variation in three key environmental conditions (air temperature, soil temperature & soil water content (SWC)) found that all three can have significant effects on soil greenhouse gas (GHG) fluxes and can affect whether a forest is a source or sink of CH_4 and N_2O (Bowden *et al.*, 1998; Luo *et al.*, 2013; Yvon-Durocher *et al.*, 2014).

Soils are not the only potential source of trace GHGs in forest ecosystems: a discrepancy between modelled CH_4 emissions derived from soil chamber data and atmospheric measurements of CH_4 over tropical wetland areas lead to the suggestion that tree stem emissions could account for the additional emissions observed above these areas (Frankenberg *et al.*, 2005). CH_4 and N_2O are produced in soils by methanogenic consortia of archaea and denitrifying bacteria respectively; the gases diffuse into soil water which is absorbed through the roots. As the water is transported up the tree stem, the gases diffuse from the xylem through the stem tissue to the atmosphere via lenticels and other structures that aid gas exchange (Carmichael *et al.*, 2014). Mesocosm studies of temperate tree and herbaceous plant species found that stem emissions reflect soil trace greenhouse gas (GHG) concentrations (Rusch and Rennenberg, 1998; Nisbet *et al.*, 2009) which has also been confirmed *in situ* for mature tree stems in a Japanese temperate floodplain forest (Terazawa *et al.*, 2007). Recent research demonstrated that tree stem CH_4 fluxes in tropical peat forests in Indonesia can account for 62-87% of total ecosystem CH_4 fluxes (Pangala *et al.* 2013).

Mesocosm studies of two common temperate tree species common to forests on free-draining soils found that processes in the rhizosphere could affect CH_4 uptake and N_2O emissions from soils by reducing soil nitrate concentrations (which is known to affect methanogenesis and denitrification) and by creating channels of greater gas diffusivity through fine root density and the maximum root depth (Fender *et al.*, 2013). Inter-species differences in physiology can affect gas transport and exchange through tree stems; in a tropical peat forest stem CH_4 fluxes were negatively correlated with wood specific density (greater density slows diffusion) and positively related to stem lenticel density as there are more pores for gas exchange to occur through (Pangala *et al.*, 2013).

As wetlands provide ideal conditions for methanogenesis, the majority of published studies have focused on temperate and tropical wetland trees. Mesocosm experiments using black alder (*Alnus glutinosa* L. Gaertn.), a typical European temperate wetland species, show that N_2O can also be emitted to the atmosphere via the tree stem pathway (Rusch and Rennenberg, 1998). Indeed, a

short-term study showed that inundation of upland forest tree species led to a spike in stem N_2O fluxes suggesting that seasonal variation in rainfall could be an important control of fluxes in these ecosystems (Machacova *et al.* 2013). Several studies investigating GHG emissions from tree stems found that emissions declined with stem height, with the largest emissions measured closer to the soil surface (Rusch and Rennenberg, 1998; Gauci *et al.*, 2010; Pangala *et al.*, 2013). Presently there is no evidence of tree stem uptake of trace GHGs however stem CH_4 fluxes recorded in Chinese upland forests showed fluxes of 0 $\mu g\ m^{-2}\ h^{-1}$ at a sampling height of 1.3-m (Wang *et al.*, 2016). However, on free-draining soils, soil water CH_4 concentrations could be low enough that ambient air CH_4 concentrations outside the stem increasingly exceed those within the stem, creating a diffusion gradient into the stems, resulting in uptake.

At present the majority of published studies into CH_4 and N_2O fluxes from European temperate forests on free-draining soils have been limited by duration or used mesocosms. These are not necessarily representative of natural conditions because abiotic conditions (precipitation and temperature) are controlled, root space is restricted and mesocosm studies generally preclude competition and interactions between individuals. Globally free-draining, aerated soils constitute 32 $Tg\ y^{-1}$ of the 632 $Tg\ y^{-1}$ annual CH_4 sink (Kirschke *et al.*, 2013). The majority of global forested area is temperate forests on free-draining soils and tree stem emissions of CH_4 could reduce or nullify the sink effect currently budgeted from these soils.

This chapter describes a study of GHG emissions from soil and tree stems in a temperate woodland on free-draining soils which aimed to test the following hypotheses:

H1) Tree stems represent a conduit for GHGs from the soil and therefore tree stems on free-draining soils will emit CH_4 and N_2O when conditions are favourable for trace GHG production.

H2) As the production of CH_4 and N_2O by specific microbial groups is regulated by SWC and temperature, soil and tree stem fluxes will vary seasonally due to changes in temperature, rainfall and SWC.

H3) Rates of evapotranspiration increase with solar radiation as plants need to transport more water to their leaves for photosynthesis, tree stem trace GHG fluxes will also increase with solar radiation as more dissolved CH_4 and N_2O is transported out of the soil in the water.

H4) Depending on their niche, tree species can have physiological and metabolic differences to gain an advantage when competing for resources. Stem fluxes will be higher from faster growing species as they have greater fine root mass, a deeper maximum root depth and produce more leaves.

4.2 Methods

4.2.1 Study site and sampling design

This study was conducted in the control plots of an existing litter manipulation experiment at Wytham Woods, an old-growth (~120 years) mixed deciduous woodland in Oxfordshire, UK. The canopy at the study site is dominated by Ash (*Fraxinus excelsior* L.), Beech (*Fagus sylvatica* L.), Sycamore (*Acer pseudoplatanus* L.) and Oak (*Quercus robur* L.*;* Fenn *et al.*, 2014). In summer 2013, 15 experimental plots measuring 25-m × 25-m each, were established in five replicate blocks. Each plot was trenched to a depth of 0.5-m, one wall was lined with plastic to limit the transfer of water and nutrients by root and hyphal networks and the trenches were then backfilled. Within each plot, four soil collars (200-mm internal diameter and 120-mm height) were embedded into the soil to 30-mm depth. The collars were installed *c*. 7.5-m from the centre of each side of the plots in July 2013. Full details of the experimental plots are given in Lopez-Sangil *et al.* (2017). As the litter manipulation treatments had only been in effect for one year and no significant effects of much longer-term treatments had been observed at the Panama site (Chapter 3), only control plots were selected for this study. In each plot, three individuals each of Ash and Sycamore were randomly selected, making a total of 24 trees. All trees were marked at 1.3-m and the girth was measured at 0.3, 0.75, 1.3, and 2-m. Only four of the five control plots were used in this study, as one of the control plots did not contain any Sycamore trees. Between February 2015 and January 2016, air temperature measured in the plots ranged from 2.1 - 24ºC (annual mean: 13.3ºC), soil temperature ranged from 3.8 -16.3ºC (annual mean: 11.9ºC) and soil water content ranged from 24.9 – 69.8% (annual mean: 44%; Fig. 4.1).

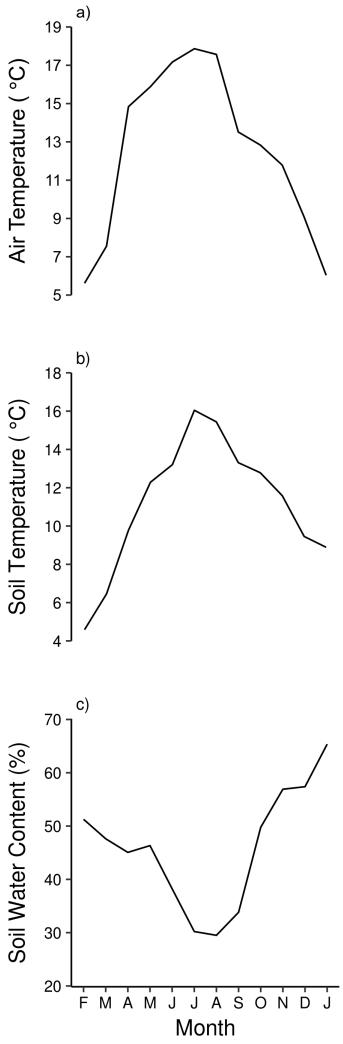

Figure 4.1 Monthly mean a) air temperature, b) soil temperature (0-6-cm depth) and c) soil water content (0-6-cm depth) recorded in a temperate woodland in Oxfordshire, UK, from February 2015 to January 2016.

Tree stem CH_4 and N_2O fluxes were sampled using the chamber design outlined in Siegenthaler *et al.* (2016). The chambers were secured to the tree stem using cam buckle straps. Play-Doh (Hasbro, United Kingdom) was used to seal fissures in Ash bark and bind the chamber to the flaky Sycamore bark, as it ensures a good seal and does not produce or absorb either of the gases studied (Siegenthaler, unpublished data). Air samples from the soil were taken by placing a Polyvinyl Chloride (PVC) lid with an inner seal of gas-tight neoprene foam on top of the soil collars. Seal integrity was demonstrated by suction on the collar after sampling. Sampling took place monthly from 16 February to 14 April 2015, then bi-monthly from 11 May to 20 October 2015 (main growing season) and then monthly from 3 November 2015 to 5 January 2016, after the majority of leaf fall had occurred. Gas samples were collected from the soil collars and from tree stem chambers at 0.3, 0.75 and 1.3-m height; 20-ml gas samples were taken by syringe at 0, 3, 6 and 10 minutes and injected into pre-evacuated 12-ml borosilicate vials (Exetainer™, LabCo Ltd,

High Wycombe, UK). From October 2015 to January 2016, tree stem fluxes were also sampled at 2-m height, after preliminary data from another study showed the potential importance of sampling higher up the stems (Wang *et al.*, 2016). Air pressure and temperature outside the stem chamber were recorded at the start of sampling using a Commeter C4141 Thermometer-Hygrometer-Barometer probe (Comet Systems, Czech Republic) and soil temperature at a 6-cm depth was recorded adjacent to the collars and the trees using a Thermapen (ETI Ltd, Worthing, UK). Volumetric soil water content at a depth of 0-6-cm depth was measured monthly using a Thetaprobe (Delta-T Devices, Cambridge, UK) calibrated to local soil conditions following the manufacturer's instructions. Data for monthly mean solar radiation and total rainfall were collected at the weather station in Wytham Woods (UK Environmental Change Network).

All samples were analysed within two weeks of collection. The CH_4 content of the samples was analysed using a Los Gatos Research FMA-200 Fast Methane Analyser (FMA; Los Gatos Research, Mountain View, CA, USA), modified to employ the 'closed loop' principle (Baird *et al.* 2010). The N_2O content of the samples was analysed using a Gas Chromatograph (Ai 94, Ellutia UK – formerly Cambridge Instruments, Ely, UK) fitted with an Electron Capture Detector.

4.2.2 Data analyses

Greenhouse gas flux data often feature a small number of extreme outliers; although these values are not necessarily due to measurement error, they are often the result of biological activity such as heartwood rot which could obscure patterns due to tree species identity or climatic variables. Consequently, the data were inspected visually and extreme outliers that lay outside of the 5^{th} - 95^{th} interquartile range were removed. As a result, 6 out of 1100 CH_4 tree stem fluxes were removed and 3 out of 227 soil chamber CH_4 fluxes were removed. 14 of 632 tree stem N_2O fluxes were determined to be outliers and 7 of 211 fluxes for soil chamber N_2O was outside the 5^{th}-95^{th} interquartile range. All statistical analyses were conducted with and without outliers and full results of the analyses including extreme outlier values are given in Appendix II. In addition, as stem CH_4 fluxes at 2-m were only measured between October 2015 and January 2016, these values were removed prior to analysis of the whole dataset. Full results for October 2015 to January 2016 including the 2-m stem height CH_4 fluxes are shown in Appendix II. Analyses including outliers for the results included in this chapter are presented in Appendix III.

All data analyses were conducted in R 3.3.2 (R Core Team, 2016) using the lme4 package for mixed effects models (Bates *et al.*, 2015). Gas fluxes were calculated for each chamber following Baird *et al.* (2010), whereby the least squares linear regression slope of the four sample concentrations is plotted against sampling time and the slope to give the gas flux in $\mu g\ m^{-2}\ h^{-1}$. Gas flux measurements were only used for further statistical analysis if the R^2 of the regression was >0.7; this cut-off point was chosen following Alm *et al.* (2007; cited in Cooper *et al.*, 2014), who noted that low fluxes (especially those near to zero) tend to have low R^2 values. Co-linearity

between environmental variables was assessed using linear models and diagnostic plots prior to modelling. The relationships between CH_4 or N_2O fluxes and climate variables were also assessed for co-linearity by calculating the variable inflation factor (VIF; *vif* function in the car package; Fox and Weisberg., 2011). As all variables had a VIF value <2, co-linearity was not deemed to be an issue for interpreting subsequent models (Zuur *et al.* 2010). Linear models were then used to examine relationships between GHG fluxes and climatic variables. Climate variables that explained a significant proportion of the variation in GHG fluxes were included as covariates in subsequent models.

The effect of sampling height and tree species on stem GHG fluxes was assessed using linear mixed effects models (*lmer* function) with sampling height, species and their interaction as fixed effects and with plot and time as random effects. As sampling height significantly influenced stem GHG fluxes, the data were subsequently analysed for each sampling height separately.

Effects of climatic variables and their interaction on soil and stem GHG fluxes were assessed using linear mixed effects models (*lmer* function) as fixed effects and with plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and p-values to check for model improvement (Pinheiro and Bates 2000). Tree species was added as a fixed effect to reach a new minimum adequate model. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. The final model fit was inspected using diagnostic plots. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ however due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

4.3 Results

4.3.1 Seasonal variation in CH_4 fluxes

There was no clear seasonal pattern in soil chamber CH_4 fluxes; although they tended to be lower in the summer, they were not significantly so (Fig. 4.2.a). Soil CH_4 fluxes decreased significantly with soil temperature ($p < 0.001$, $r^2 = 0.058$, $\chi^2 = 11.9$; Fig. 4.3.b) and there was also a significant interaction between soil temperature × SWC ($p < 0.01$, $r^2 = 0.060$, $\chi^2 = 12.5$) and soil temperature × air temperature ($p < 0.01$, $r^2 = 0.063$, $\chi^2 = 13$).

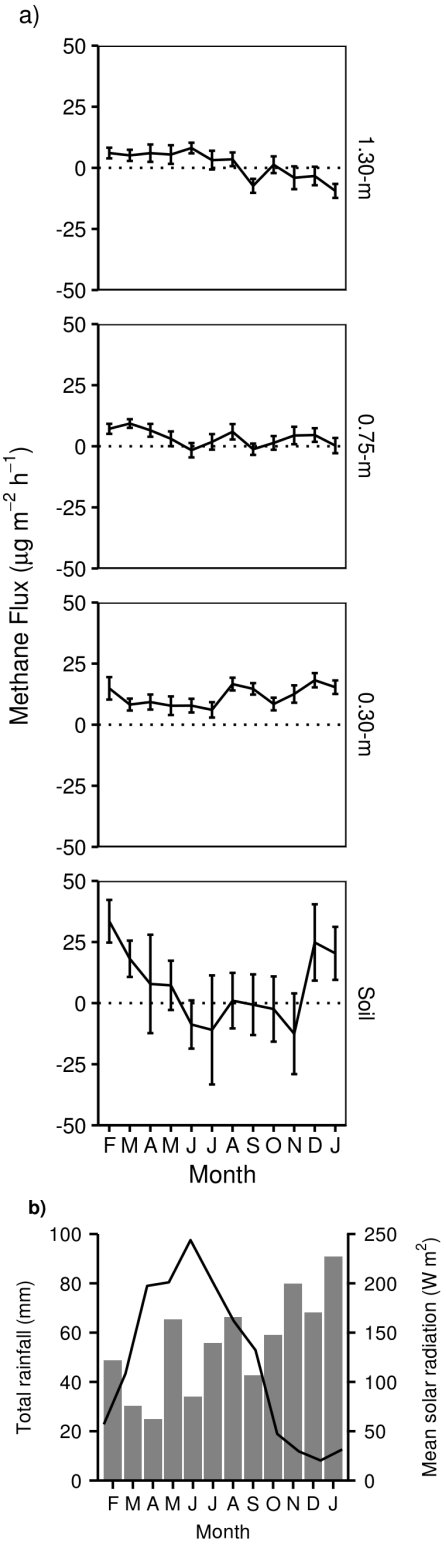

Figure 4.2 a) Seasonal patterns of methane (CH_4) fluxes from tree stems and soil in a temperate woodland on free-draining soil in Oxfordshire, UK, showing combined monthly mean stem fluxes, measured at 0.3-m, 0.75-m and 1.3-m from Ash and Sycamore trees, and monthly mean soil fluxes measured over chambers between February 2015 and January 2016; error bars show the standard error means for *n* = 4; b) bars show the total monthly rainfall and the line shows mean solar radiation at Wytham Woods.

Tree stem CH_4 fluxes were not related to air temperature, soil temperature, SWC or total monthly rainfall but CH_4 fluxes declined significantly with sampling height ($p < 0.0001$, $r^2 = 0.061$, $\chi^2 = 55.2$). Tree stem CH_4 fluxes at 0.3-m showed strong seasonal differences, whereby mean CH_4 fluxes between August and December 2015 were higher than those between February and July 2015 ($p < 0.05$, $r^2 = 0.074$, $\chi^2 = 4.82$; Fig. 4.2.a). There was also a marginally significant seasonal difference at 1.3-m stem height where CH_4 fluxes decreased between February 2015 and January 2016 ($p < 0.1$, $r^2 = 0.133$, $\chi^2 = 2.88$). There was no clear pattern of seasonality for stem CH_4 fluxes at any other sampling height.

Stem CH_4 fluxes at different sampling heights showed varied relationships with climatic variables: there was a marginal negative effect of rising soil temperatures on stem CH_4 fluxes at 0.75-m ($p < 0.1$, $r^2 = 0.162$, $\chi^2 = 2.76$; Fig. 4.3.b) whereas stem CH_4 fluxes were positively related to solar radiation at 1.3-m ($p < 0.05$, $r^2 = 0.0.133$, $\chi^2 = 5.74$) and marginally at 0.3-m ($p < 0.1$, $r^2 = 0.052$, $\chi^2 = 3.08$). At 1.3-m height, there was a significant solar radiation × rainfall interaction ($p < 0.05$, $r^2 = 0.138$, $\chi^2 = 8.03$).

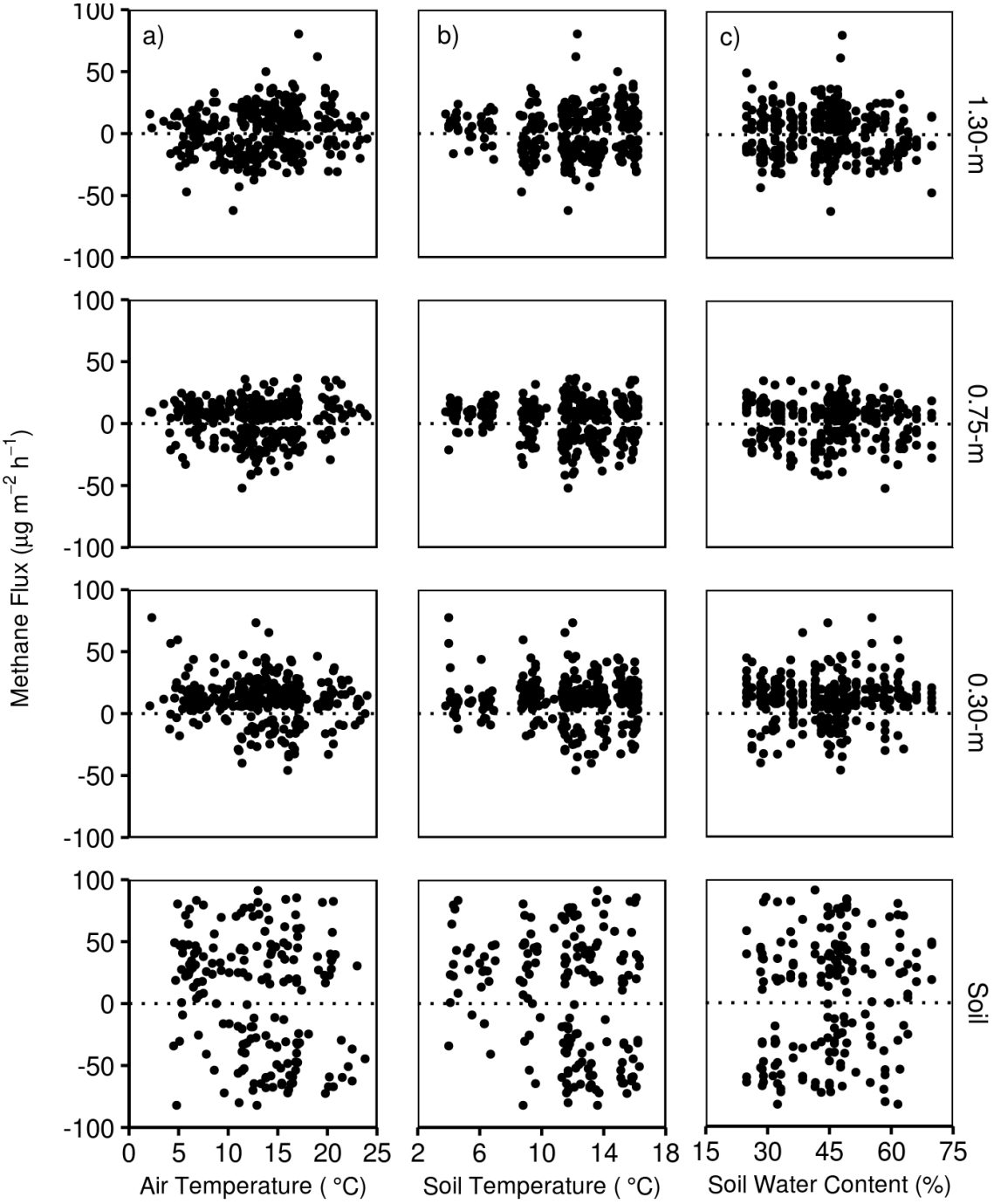

Figure 4.3 Scatter plots of the relationship between methane (CH_4) fluxes from soil chambers or tree stems at 0.3-m, 0.75-m and 1.3-m sampling height and a) air temperature, b) soil temperature and c) soil water content (SWC) in a temperate woodland on free-draining soil in Oxfordshire, UK between February 2015 and January 2016. Tree stem CH_4 fluxes shown are pooled fluxes from both Ash and Sycamore trees.

4.3.2 Soil CH_4 flux

Soil chamber CH_4 fluxes were negative for much of the year, declining from emission to uptake in the spring and summer before transitioning back to emission in the winter months of December (Fig. 4.4) and January which coincided with the highest mean SWC values. The median soil CH_4 flux between February 2015 and January 2016 was 18.3 µg m^{-2} hr^{-1} and fluxes ranged from -203 µg m^{-2} hr^{-1} to 170 µg m^{-2} hr^{-1}. Unlike tree stem CH_4 fluxes, there was no significant or marginal change over time. Overall the soils were a small source between February 2015 and January 2016 with a mean flux of 4.87±4.14 µg m^{-2} hr^{-1}.

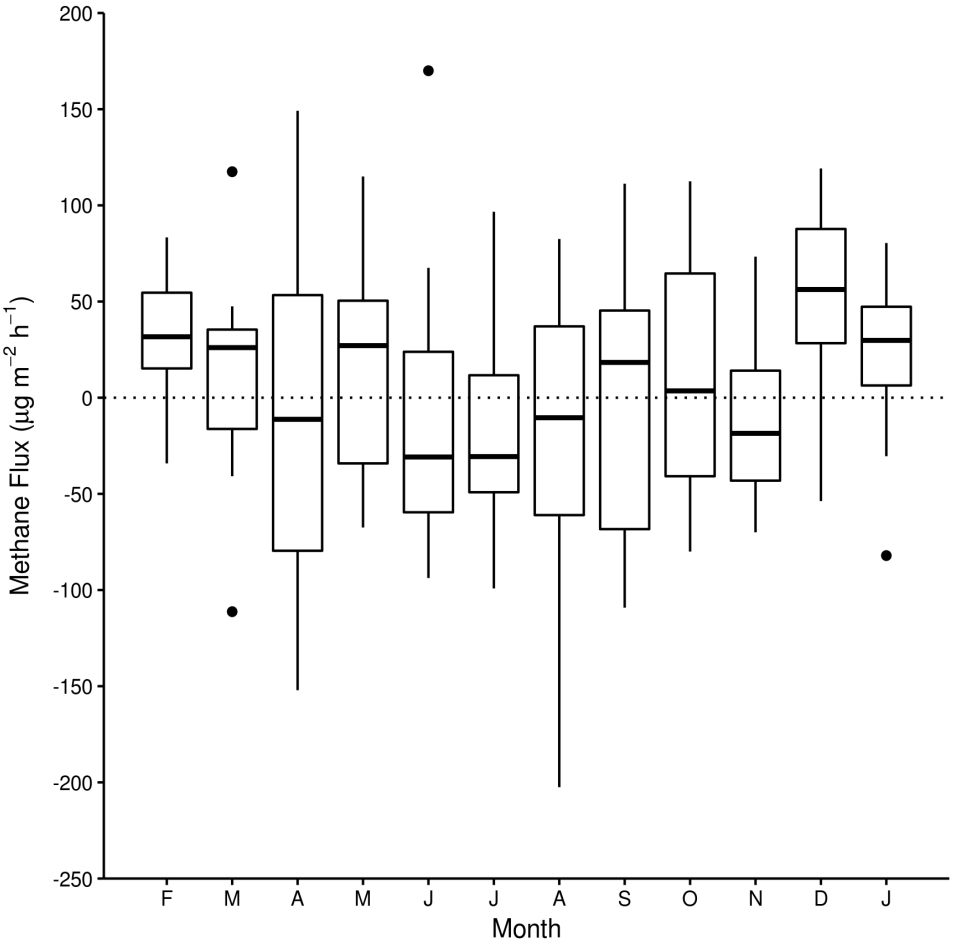

Figure 4.4 Monthly ranges of methane (CH_4) fluxes measured over soil chambers in a temperate woodland on free-draining soil in Oxfordshire, UK, between February 2015 and January 2016. Ranges represent four replicates.

4.3.3 Tree stem CH_4 fluxes

Tree stem CH_4 fluxes varied with sampling height, from mostly positive at 0.3-m to mostly negative at 1.3-m. Ash stem CH_4 fluxes were marginally lower than Sycamore stem CH_4 fluxes at 0.3-m ($p < 0.1$, $r^2 = 0.054$, $\chi^2 = 3.64$) but slightly higher than Sycamore stem CH_4 fluxes at 0.75-m ($p < 0.1$, $r^2 = 0.173$, $\chi^2 = 2.82$). The range of CH_4 fluxes was greater from Sycamore stems at each height (Table 4.1).

Stem sampling position(m)	**Flux range (µg m^{-2} hr^{-1})**		**Median flux (µg m^{-2} hr^{-1})**	
	Ash	Sycamore	Ash	Sycamore
0.3	-35 – 47.5	-32.9 – 56.7	10.8	12.9
0.75	-41.7 – 35.8	-52.1 – 36.7	7.50	6.67
1.3	-42.9 – 62.1	-62.1 – 37.1	4.17	5.42

Table 4.1 Range and median of CH_4 fluxes recorded at 0.3-m, 0.75-m and 1.3-m from Ash and Sycamore trees in Wytham Woods between February 2015 and January 2016.

Ash and Sycamore stem CH_4 fluxes at 0.3-m were generally positive. Mean CH_4 flux was 9.29 ±1.31 µg m^{-2} hr^{-1} from Ash stems compared to 12.5 ±1.04 µg m^{-2} hr^{-1} from Sycamore stems (Fig. 4.5). At 0.75-m, Ash stem median CH_4 fluxes were consistently positive whereas median Sycamore stem CH_4 fluxes were highly variable, particularly between July and November 2015. As a result, mean CH_4 flux from Ash stems at 0.75-m height was 3.99±1.15 µg m^{-2} hr^{-1}, which was more than double that of Sycamore (1.88±1.27 µg m^{-2} hr^{-1}). At 1.3-m, both species showed a trend of declining CH_4 fluxes over the sampling period which was more pronounced in Ash. At this height the mean CH_4 flux from Ash stems was very slightly negative (-0.37±1.35 µg m^{-2} hr^{-1}) whereas mean CH_4 flux from Sycamore stems was 2.90±1.37 µg m^{-2} hr^{-1}.

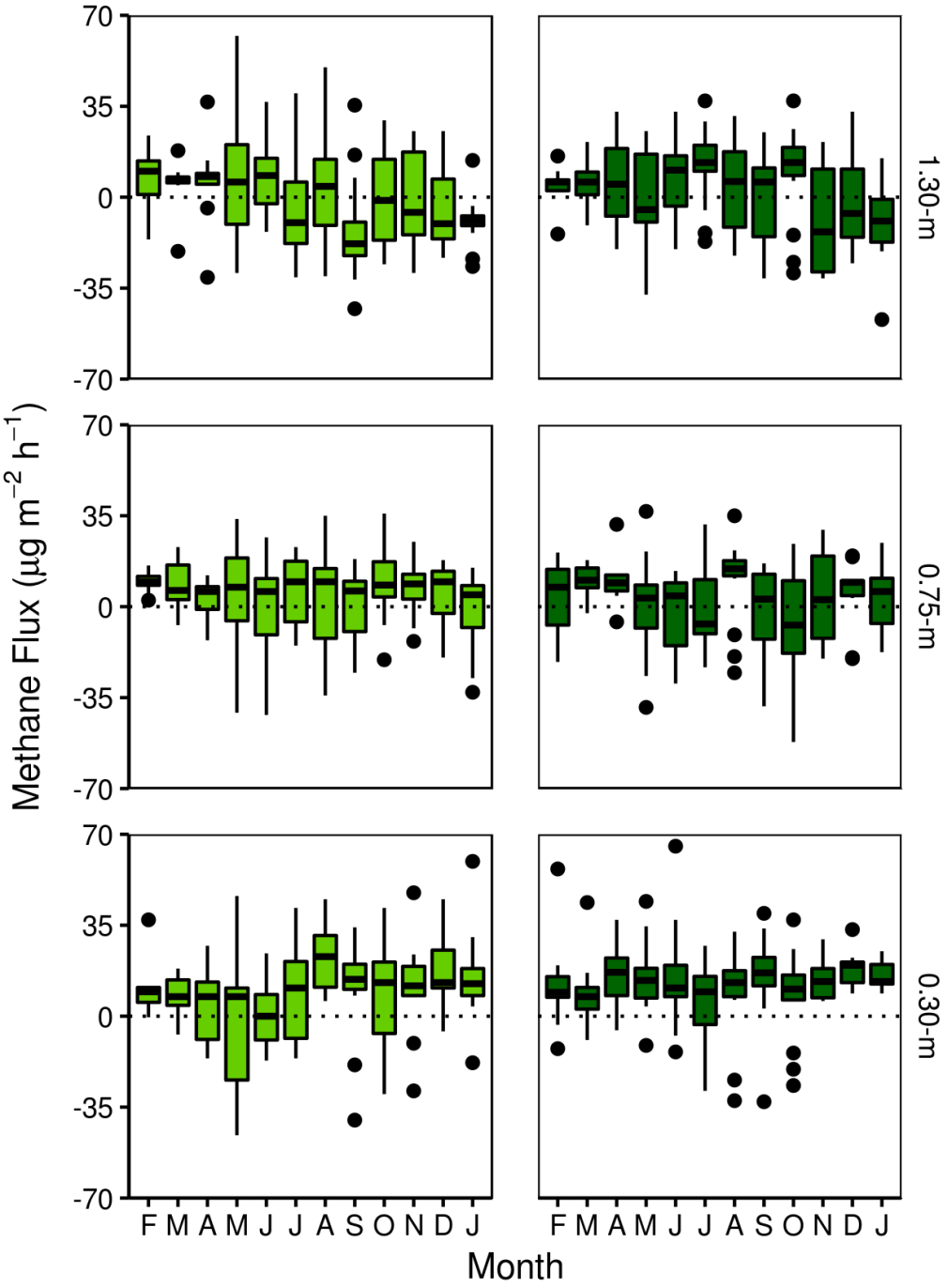

Figure 4.5 Monthly ranges of methane (CH_4) fluxes from tree stems in a temperate woodland on free-draining soil in Oxfordshire, UK, showing stem fluxes measured at 0.3-m, 0.75-m and 1.3-m in two common species: Ash (pale green) and Sycamore (dark green) between February 2015 and January 2016. Ranges are based on four replicates per species.

4.3.4 Seasonal variation in N_2O flux

Although soil N_2O fluxes tended to be higher during months with high rainfall (Fig. 4.6.a), there were no clear seasonal patterns in soil N_2O fluxes, nor was there a significant difference between seasons. There was no significant relationship between soil N_2O fluxes and any of the measured climatic variables.

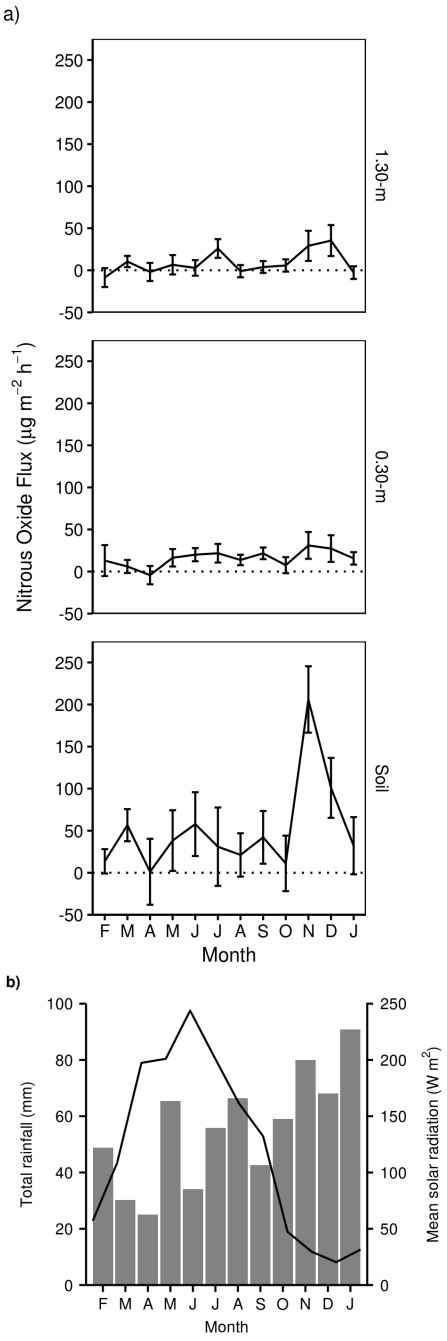

Figure 4.6 a) Seasonal patterns of pooled nitrous oxide (N_2O) fluxes from tree stems and soil in a temperate woodland on free-draining soil in Oxfordshire, UK, showing monthly mean stem fluxes measured at 0.3-m and 1.3-m, and monthly mean soil fluxes measured over chambers between February 2015 and January 2016; error bars show the standard error means for n = 4; b) bars show the total monthly rainfall and the line shows mean solar radiation at Wytham Woods.

Similarly, there was no seasonal pattern for tree stem N_2O fluxes (Fig. 4.6.a) but there was a marginally significant difference in stem N_2O fluxes at 0.3 and 1.3-m ($p < 0.1$, $r^2 = 0.019$, $\chi^2 = 2.95$) and stem N_2O fluxes at different heights showed varying responses to climatic variables. Air temperature and SWC had no effect on tree stem N_2O fluxes at 0.3-m or 1.3-m height but tree stem N_2O fluxes at 0.3-m increased with soil temperature ($p < 0.1$, $r^2 = 0.012$, $\chi^2 = 3.73$; Fig. 4.7.b). There was no relationship between soil temperature and stem fluxes at 1.3-m.

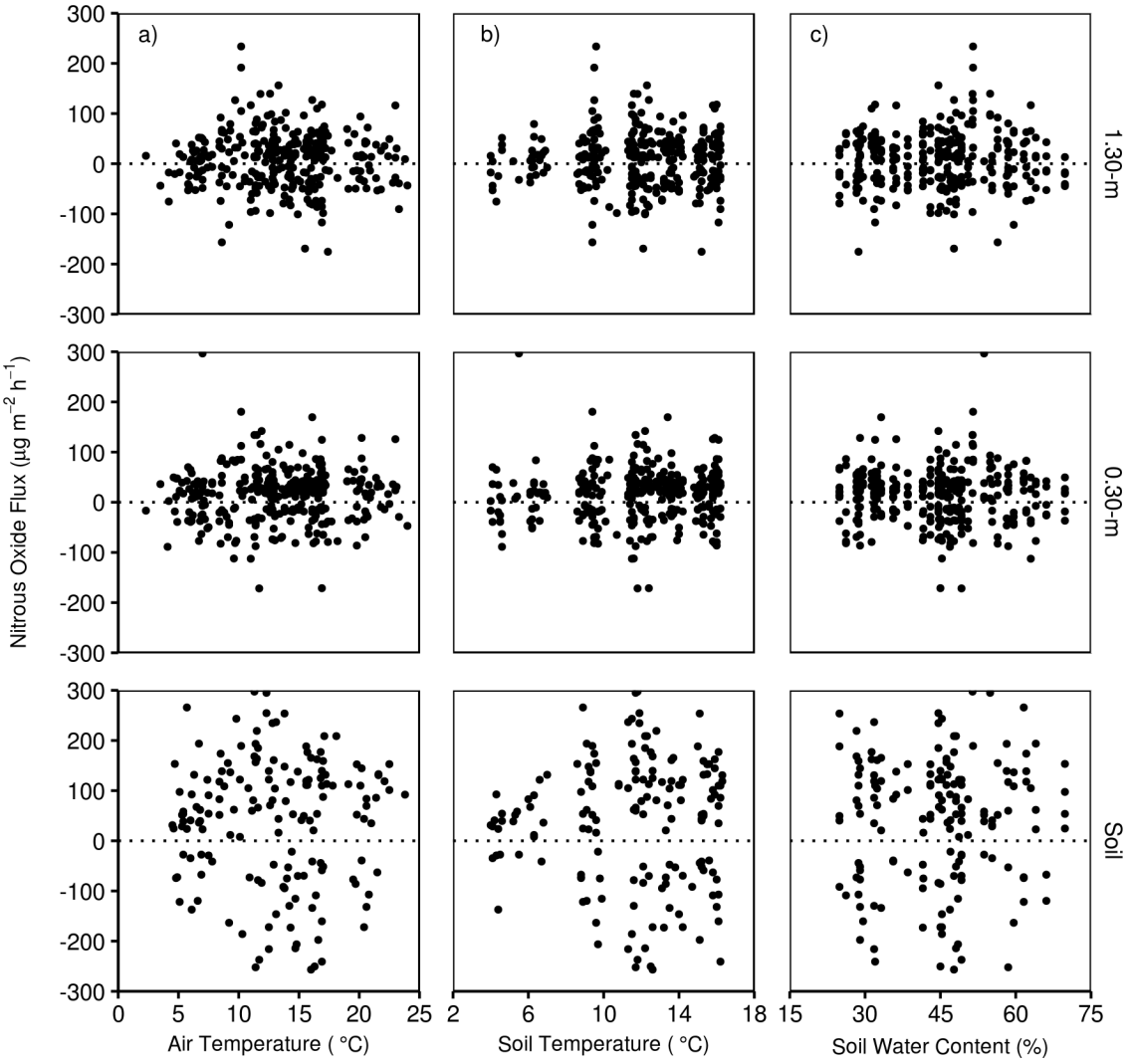

Figure 4.7 Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from soil chambers or tree stems at 0.3-m and 1.3-m and a) air temperature, b) soil temperature and c) soil water content (SWC) in a temperate woodland on free-draining soil in Oxfordshire, UK between February 2015 and January 2016. Tree stem N_2O fluxes shown are pooled from Ash and Sycamore trees.

4.3.5 Soil N_2O fluxes

Soil N_2O fluxes were mostly positive throughout the study, with the majority of uptake between April and October 2015. The sudden increase in N_2O fluxes in November 2015 coincided with increased rainfall at Wytham Woods (Fig. 4.6). Over the course of the study N_2O fluxes ranged from -603 µg m^{-2} hr^{-1} to 575 µg m^{-2} hr^{-1} with a median flux of 60 µg m^{-2} hr^{-1}. The mean soil N_2O flux over the 12-month study was 42.5±13 µg m^{-2} hr^{-1}.

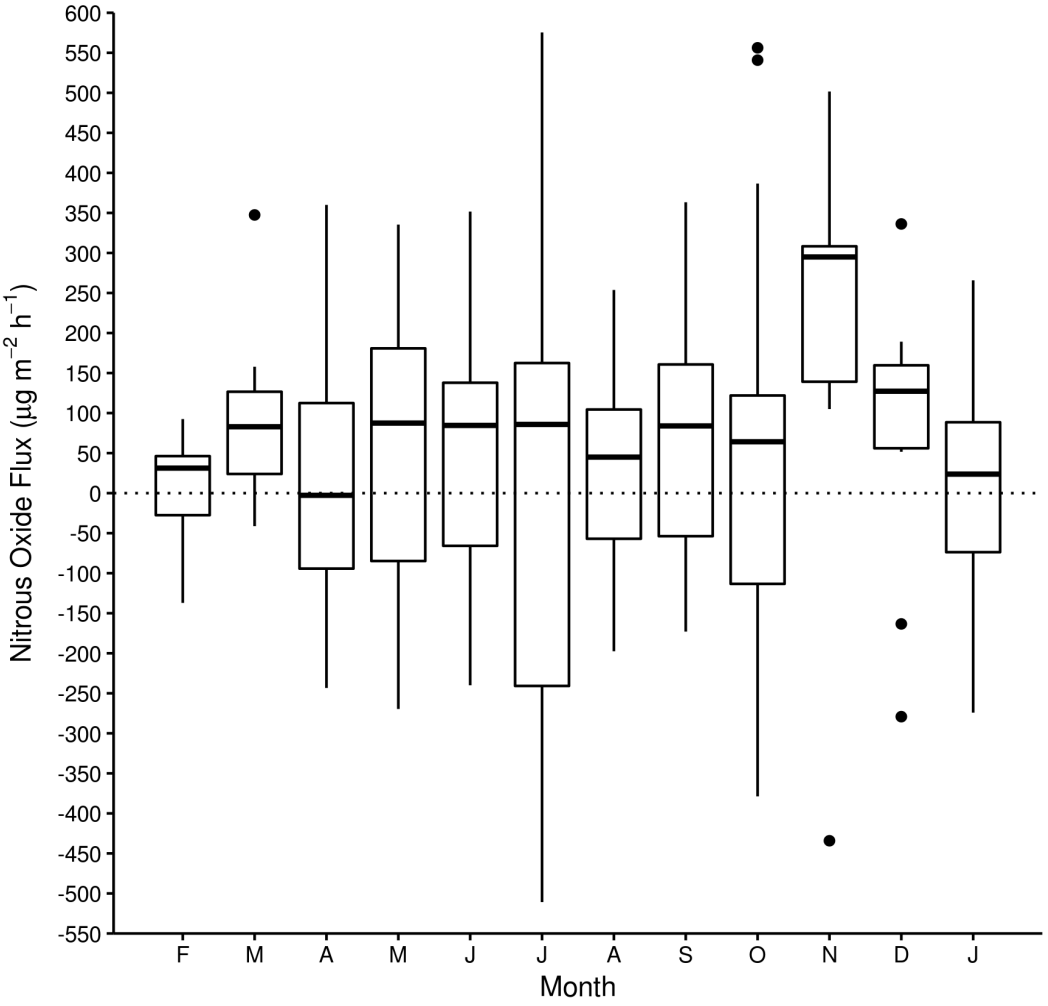

Figure 4.8 Monthly ranges of nitrous oxide (N_2O) fluxes measured over soil chambers in a temperate woodland on free-draining soil in Oxfordshire, UK, between February 2015 and January 2016. Ranges are based on four replicates.

4.3.6 Tree stem N_2O fluxes

Similar to the pattern observed for CH_4 fluxes, tree stem N_2O fluxes also decreased with stem height ($p < 0.1$, $r^2 = 0.019$, $\chi^2 = 2.95$) but there was no difference between species at either 0.3-m or 1.3-m sampling height. The range of N_2O fluxes at each sampling height was smaller for Ash stems than for Sycamore (Table 4.2).

Stem sampling position (m)	**Flux range (µg m^{-2} hr^{-1})**		**Annual median flux (µg m^{-2} hr^{-1})**	
	Ash	Sycamore	Ash	Sycamore
0.3	-113 – 142	-112 – 170	20.2	23.8
1.3	-157 – 128	-175 – 192	15.8	12.1

Table 4.2 Range and median of N_2O fluxes recorded at 0.3-m and 1.3-m from Ash and Sycamore trees in Wytham Woods between February 2015 and January 2016.

At 0.3-m sampling height, stem fluxes of N_2O were generally positive although the median N_2O flux for both species was negative in April 2015 (Fig. 4.9). Mean stem N_2O fluxes at 0.3-m were 12.8 ±3.89 µg m^{-2} hr^{-1} for Ash and 17.5 ±3.83 µg m^{-2} hr^{-1} for Sycamore. Stem N_2O fluxes at 1.3-m stem height were a lot more variable throughout the sampling period. At 1.3-m, mean stem N_2O flux was 12.1 ±3.93 µg m^{-2} hr^{-1} for ash and 3.02 ±4.64 µg m^{-2} hr^{-1} for Sycamore.

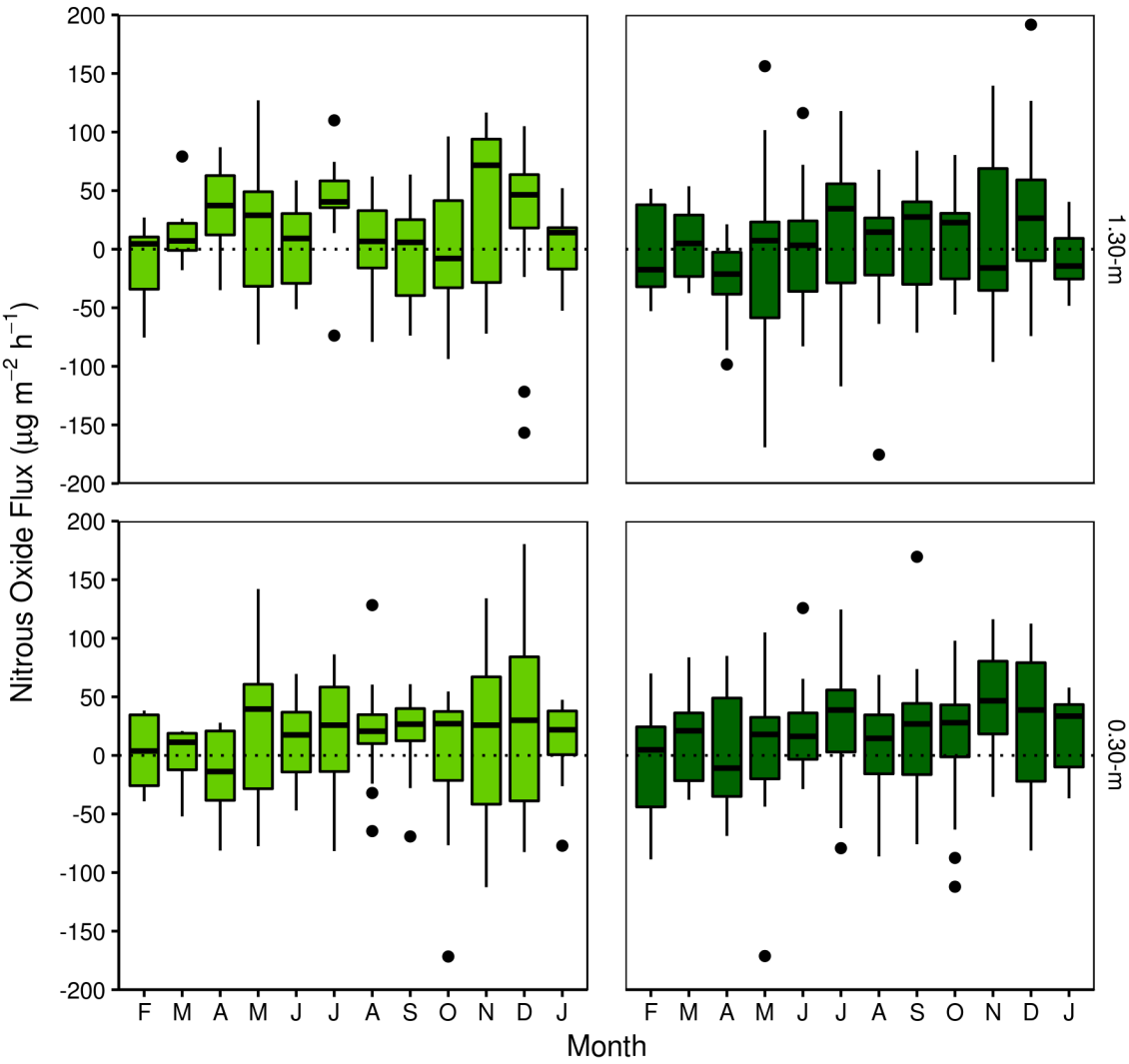

Figure 4.9 Monthly ranges of nitrous oxide (N_2O) fluxes from tree stems in a temperate woodland on free-draining soil in Oxfordshire, UK, showing stem fluxes measured at 0.3-m and 1.3-m in two common species: Ash (pale green) and Sycamore (dark green) between February 2015 and January 2016. Ranges are based on four replicates per species.

4.4 Discussion

This study demonstrates that temperate trees on free-draining soils can act as sources and sinks of CH_4 and N_2O. Although emissions of trace GHG were measured at the bottom of tree stems, fluxes of CH_4 and N_2O declined with sampling height and were mostly negative at 1.3-m and 2-m. These novel findings on height variation in stem CH_4 and N_2O fluxes likely resulted from the interaction of abiotic and biotic factors that can affect production of trace GHGs in soils and their transport from soils to atmosphere via tree stems.

4.4.1 Seasonal patterns and abiotic controls of GHG fluxes

Although there were no clear seasonal patterns in soil or tree stem fluxes of CH_4 and N_2O, peaks in the fluxes of both GHGs from the soil coincided with periods of high SWC (Fig. 4.2 and 4.6). SWC is the strongest driver of variability in forest soil GHG fluxes and as the majority of tree stem CH_4 and N_2O emissions are derived from microbial activity in the soil (Rusch and Rennenberg, 1998; Gauci *et al.*, 2010), it is reasonable to assume that changes in soil CH_4 and N_2O fluxes with SWC would also affect CH_4 and N_2O fluxes from tree stems. CH_4 oxidation rates decline and rates of methanogenesis and denitrification increase with SWC because soil oxygen concentrations decrease as the soil becomes increasingly saturated, which induces favourable conditions for obligate anaerobe microbial communities that produce greenhouse gases (Borken *et al.*, 2006; Luo *et al.*, 2013). In a German spruce forest soil, CH_4 uptake was greatest (-70 μg CH_4 m^{-2} h^{-1}) in summer and declined significantly as SWC increased with precipitation (Luo *et al.*, 2013); similarly soil CH_4 fluxes in this study were highest during the period of greatest rainfall (Fig. 4.2). The majority of CH_4 oxidation occurs near the soil surface therefore soil CH_4 fluxes will be more responsive to changes in SWC that could affect CH_4 oxidation and methanogenesis. CH_4 fluxes from tree stems are not so readily affected by change in SWC as the CH_4 is drawn into the tree in soil water from deeper in the soil profile bypassing the methanotrophic topsoil.

The increase in soil and tree stem CH_4 and N_2O fluxes between September 2015 and January 2016 (Fig. 4.2.a & 4.6.a) could be partly due to leaf fall in October and November 2015. Leaf litter not only provides source material for soil chemical processes but can also affect soil moisture, soil temperature and rates of gas exchange between soils and the atmosphere (Sayer, 2006). A combination of higher SWC and a thicker layer of litter following leaf fall at the end of the growing season could retard the diffusion of oxygen into the topsoil, resulting in anaerobic conditions that favour methanogenesis and denitrification (Dong *et al.*, 1998; Dubbs and Whalen, 2010). In wetland soils, competition for acetate between methanogenic archaea and other microbes becomes the main control of CH_4 production when the soils are saturated (Brooks Avery *et al.*, 2003; Teh *et al.*, 2008). Hence, higher soil CH_4 fluxes during December 2015 in this study (Fig. 4.4) could be the result of increased acetate production during litter decomposition at high SWC

(Fig. 4.1). Furthermore, increased nitrogen mineralization rates after leaf fall (Rosenkranz *et al.*, 2006), coupled with SWC close to the 70-80% moisture optima for N_2O production (Butterbach-Bahl *et al.* 2013) could explain the peak in N_2O fluxes during November 2015 in this study (Fig. 4.8 & Fig. 4.9). Similarly, higher fluxes of CH_4 and N_2O measured from soils and tree stems in February and March 2015 (Fig. 4.2.a & Fig. 4.6.a) likely arose as a legacy of litterfall and high SWC in winter 2014/15.

The lack of clear relationships and seasonal patterns for N_2O fluxes can be explained by difficulties in aligning sampling frequency and timing with maximum denitrification rates. For example, mesocosm studies of N_2O reported peak emissions from temperate wetland saplings 24 hours after rewetting (Machacova *et al.* 2012) which was followed by a rapid decline to ambient levels. The high degree of spatial and temporal variability in soil and stem N_2O fluxes in this temperate woodland on free-draining soils is similar to the patterns observed in a lowland tropical rainforest on free-draining soils (Fig. 3.9 & Fig. 3.10, Chapter 3). The consistency of this variability between climatic zones shows how areas with disproportionately high denitrification rates (hotspots) and sampling within short time periods of high reaction rates (hot moments) could affect the observation of seasonal patterns in soil and tree stem N_2O fluxes (McClain *et al.*, 2003).

There was no relationship between air temperature and soil or tree stem CH_4 and N_2O fluxes in Wytham which is unexpected as air and soil temperatures varied similarly throughout the year (Fig. 4.1). Mean air temperature had no effect on soil trace greenhouse gas fluxes in a range of temperate forests on free-draining soils, wetlands and uplands across Europe (Brumme *et al.*, 1999; Gunderson *et al.*, 2012) and as the majority of natural terrestrial CH_4 and N_2O is produced by soil microorganisms (IPCC, 2013), changes in soil temperature are more likely to affect soil and tree stem CH_4 and N_2O fluxes. In the present study, soil and tree stem CH_4 fluxes declined with increasing soil temperature whereas tree stem N_2O fluxes at 0.3-m tended to increase. Seasonal patterns in soil GHG fluxes, although not significant, also tracked soil temperature, as mean soil fluxes of CH_4 decreased between April and July 2015 (Fig. 4.2.a) as soil temperatures rose, whereas mean soil N_2O increased (Fig. 4.6.a). Similar patterns have been observed along a climatic gradient between Sweden and Italy, where CH_4 oxidation decreased with increasing soil temperature but N_2O emissions increased (Gunderson *et al.*, 2012). Methanotrophy, methanogenesis and denitrification are all microbial processes and soil temperature variation can influence these processes by modifying metabolic and gas diffusivity rates (King and Adamsen, 1992; Butterbach-Bahl *et al.*, 2013). Soil temperature is only a significant control of these process once a threshold temperature has been exceeded. Microbial activity is strongly constrained at temperatures below c. 10ºC and accordingly, CH_4 oxidation rates in a temperate forest in the USA declined at soil temperatures between -5ºC and 10ºC due to significantly reduced microbial activity in the uppermost soils but there was no effect of temperature on oxidation rates between 10ºC and 25ºC (Castro *et al.*, 1995). This may explain why the range of soil and stem CH_4 fluxes increased as soil

temperatures rose from 3°C to 10°C in the present study (Fig. 4.3.b). Temperature responses of N_2O emissions in laboratory studies are bell-shaped, peak N_2O emissions have a temperature optima of ~20-35°C however such responses are not found *in situ* (Barnard *et al.*, 2005). Soil temperatures may not have reached the 20°C optima but this could also explain the increasing range of soil and stem N_2O fluxes between 3°C and 12°C (Fig. 4.7.b).

The seasonal patterns of soil and tree stem GHG fluxes in this study can largely be explained by the combination of SWC, soil temperature and peak litterfall at the end of the growing season. In the early stages of the sampling campaign (February and March 2015; Fig. 4.2 & 4.6), there was a short burst of increased methanogenesis and denitrification as soil temperature (and consequently reaction rates) increased in the water-saturated soils. Between April and September 2015 (Fig. 4.1), SWC declined as temperatures rose; consequently, soil methanotrophy increased and denitrification decreased, resulting in soil CH_4 uptake, diminished soil N_2O emissions and lower tree stem fluxes of CH_4 and N_2O in the summer months (Fig. 4.2.a & Fig.4.6.a). From August to December 2015, tree stem fluxes of CH_4 and N_2O rose steadily at multiple sampling heights, with the highest mean CH_4 fluxes in December 2015 and the highest mean N_2O fluxes in November 2015 (Fig. 4.2.a & Fig.4.6.a); this increase in stem GHG fluxes coincided with rising SWC (Fig. 4.1) during the period of peak litterfall. High SWC likely offset lower temperatures at this time of year, such that even small increases in acetate production and nitrate release during decomposition would lead to greater emissions of CH_4 and N_2O from soils and tree stems.

The outlier values for both CH_4 and N_2O fluxes (Appendix III) show the need to consider all trace GHG fluxes recorded during long-term monitoring in forest ecosystems. The presence of 'hotspots' in the soil of larger communities of methanogens and denitrifying bacteria, coupled with 'hot moments' where air/soil temperatures and SWC reach the optima for GHG production must be taken into account in ecosystem trace GHG budgets by including these outlier flux values. In the present study, the highest soil CH_4 and stem CH_4 outliers (at 0.30-m and 1.30-m) were measured at soil temperatures of 10°C (App. III Fig. 2b), above which temperature no longer significantly limits soil microbial activity (Castro *et al*., 1995). Similarly, the largest outliers in soil and stem (0.30-m) N_2O fluxes were measured as SWC approached the 70% optimum observed by Butterbach-Bahl *et al*. (2013; App. III Fig. 6c). However, the largest measured CH_4 flux of 252 μg m^{-2} h^{-1} from a Sycamore stem (at 0.75-m) occurred in August at a surface SWC of almost 20% and a soil temperature at 6-cm depth above 15°C. This discrepancy demonstrates the limitations of applying the results from laboratory and mesocosm studies to field experiments, as well as the need to build long-term datasets in forests on free-draining soils.

The broad seasonal pattern suggests that SWC is a more important abiotic control of CH_4 and N_2O fluxes in Wytham Woods than soil temperature (Topp and Pattey, 1997; Guckland *et al.*,

2009; Butterbach-Bahl *et al.*, 2013) but an understanding of interactions among soil water content, soil temperature, and decomposition processes are required to fully interpret seasonal patterns.

4.4.2 Species effects

Tree species can affect soil CH_4 and N_2O fluxes by modifying soil chemistry and structure, which affect microbial activity and gas transport through the soils and into the trees. Variation in fine root biomass, litter mass and soil nitrate concentrations can all influence soil N_2O and CH_4 fluxes under different tree species (Wang *et al.*, 2013). Results from the present study demonstrate differences in CH_4 emissions from Ash and Sycamore stems but N_2O fluxes did not vary between species.

Ash trees are widespread across Europe and as such have been used in several mesocosm studies to investigate how species affect soil CH_4 and N_2O fluxes via differences in root biomass. Soil CH_4 uptake in mesocosms with Ash saplings was almost three times greater than rootless control mesocosms and significantly higher than in mesocosms with Beech saplings, due to the greater fine root biomass in ash mesocosms (Fender *et al.*, 2013a). Greater fine root biomass enhances inter-connectivity between soil pore spaces leading to increased rates of gas diffusion. As gas diffusion rates increase, a greater proportion of soil CH_4 is likely to reach the aerated topsoil where the majority of methanogenesis occurs, increasing soil CH_4 uptake. This in turn reduces overall soil CH_4 concentrations, decreasing stem CH_4 fluxes. The marginal difference in stem CH_4 fluxes, seen at 0.3-m where Ash stem CH_4 fluxes were larger than those from Sycamore and 0.75-m, where Ash stem CH_4 fluxes remained largely positive unlike the more variable Sycamore stem CH_4 fluxes (Fig. 4.5) could be due to differences between species in litter decomposition rates, which could decrease stem CH_4 fluxes. Resistance of leaf litter to mineralization can affect soil CH_4 and N_2O fluxes as it reduces nutrient availability. Ash litter decomposes more slowly than Sycamore litter (Aubert *et al.*, 2010), therefore acetate concentrations should be lower under Ash trees reducing soil and stem CH_4 fluxes however this was not seen at Wytham. A mesocosm study of rhizospheric changes in soil chemistry under different species found that N_2O efflux was reduced by 94% at ambient nitrate availability in Ash saplings compared to Beech, yet when a mixture of Ash and Beech saplings was grown under the same conditions, the reduction in N_2O efflux was half of the mono-culture mesocosms (Fender *et al.*, 2013b). Given the mixture of canopy species at Wytham Woods, it is possible that potential effects of any one species on soil (and by extension tree stem) CH_4 and N_2O fluxes could be mitigated by the presence of other species.

Soil structural changes from differences in fine root biomass are important controls of CH_4 and N_2O fluxes however species-specific effects on soil chemistry, such as pH and Carbon:Nitrogen (C:N) ratios, may be equally important. Soil pH and C:N ratios are largely controlled by soil organic matter much of which is provided by litterfall. Trees can alter soil pH by modifying the availability of anions in the soil, however these effects are limited to the upper 0-10-

cm of soils (Augusto *et al.*, 2002; Hagen-Thorn *et al.*, 2004). As soils become more acidic, conditions become suboptimal for methanotrophs, reducing rates of methanotrophy (Hanson and Hanson, 1996) and increasing CH_4 emissions. Forest soil N_2O fluxes are also positively correlated with soil pH, as soil pH <6 inhibits the activity of the nitrous-oxide reductase enzyme favouring N_2O production over complete denitrification to N_2 (Šimek and Cooper, 2002). A separate but concurrent study at Wytham Woods demonstrated significantly lower soil pH under Sycamore compared to Ash trees (Medina-Barcenas *et al.* unpublished data). Mean CH_4 and N_2O fluxes from Ash stems were lower than those from Sycamore stems at 0.3-m. Mean CH_4 fluxes at 1.3-m were also lower from Ash than Sycamore stems. Therefore it is possible that part of the differences in stem CH_4 and N_2O fluxes between species at Wytham Woods could arise from changes in soil pH.

Species induced changes in soil C:N ratios can affect methanotrophy and denitrification by disrupting enzyme activity in the soil. A study of soil fluxes across Europe found that CH_4 fluxes were positively related to the C:N ratio of the soil (Gunderson *et al.*, 2012). The active site of the methane mono-oxygenase enzyme is able to process ammonium and CH_4, so as N availability increases, the competition between the two molecules reduces CH_4 oxidation (Hanson and Hanson, 1996). Soil N_2O fluxes are highest at low C:N ratios as there is a greater availability of N; this in turn increases nitrate concentrations, leading to higher N_2O emissions (Gunderson *et al.*, 2012). A concurrent study at Wytham Woods demonstrated that the soil C:N ratio was much lower under Ash compared to Sycamore trees (Medina-Barcenas *et al.* unpublished data), which could explain the marginally smaller CH_4 stem fluxes from Ash trees at 0.3-m and 1.3-m compared to Sycamore (Table 4.1). The lack of inter-species differences in N_2O fluxes could be due to the species mixture present in the plots which may have prevented localised effects of Ash or Sycamore litter.

Several studies have reported inter-species variation in stem CH_4 and N_2O fluxes (Covey *et al.*, 2012; Machacova *et al.*, 2013 and Pangala *et al.*, 2013). Stem lenticel density, wood specific density and stem diameter all have significant effects on stem CH_4 fluxes. Wood specific density and stem diameter are both negatively correlated with tree stem CH_4 fluxes in tropical wetland forests (Pangala *et al.*, 2013): as woody tissue becomes more dense with age it is harder to exchange internal gases with the atmosphere and stem diameter is correlated with age. There is little difference in the wood specific density of Ash (0.53 g cm^{-3}) and Sycamore (0.49 g cm^{-3}; Wiemann *et al.*, 2007), which may partly explain the lack of significant variation in stem CH_4 and N_2O fluxes between species in the present study.

4.4.3 Tree stems and ecosystem GHG exchange

By sampling at different heights, this study demonstrates that trees in this deciduous woodland mainly emit CH_4 and N_2O at the bottom of their stems (0.3-m) but there is uptake of GHGs above ~1.3-m stem height which could have important impacts on ecosystem exchange of both gases. As the majority of the woody surface area of mature tree stems is above 1.3-m stem height, CH_4 fluxes

measured at 2-m may be more representative of the contribution of trees to biosphere-atmosphere exchange of trace greenhouse gases. Previous studies in the Amazon (Pangala *et al.*, 2013), China (Wang *et al.*, 2016) and the USA (Megonigal *et al.*, 2016) all reported decreasing CH_4 fluxes with sampling height across multiple species but they did not demonstrate stem uptake of CH_4.

Solar radiation was hypothesised to affect stem GHG fluxes via water transport through evapotranspiration during photosynthesis. Stem CH_4 fluxes had a marginally negative relationship with solar radiation at 0.3-m but was significantly positively related to stem CH_4 fluxes at 1.3-m. Peak mean stem CH_4 flux at 1.3-m at Wytham occurred in June 2015 which coincided with the highest monthly mean solar radiation (Fig. 4.2.a). However, the increase in CH_4 fluxes at 1.3-m is surprising because June 2015 was one of the driest months during the study (Fig. 4.1) and CH_4 fluxes declined at 0.3-m and 0.75-m. The increase at 1.3-m in stem CH_4 fluxes with solar radiation could be due to higher summer temperatures increasing the rates of heartwood rot and not an effect of elevated transpiration. For example, in upland deciduous trees in the USA, heartwood CH_4 concentrations at 1.3-m were three times higher in the summer than the spring (Covey *et al.*, 2012). As soil CH_4 concentrations declined, stem CH_4 fluxes at 0.3-m and 0.75-m where there is less or no heartwood rot would decrease, whereas stem CH_4 fluxes at 1.3-m would increase.

The patterns in stem CH_4 fluxes with sampling height reversed between August 2015 and January 2016: stem CH_4 fluxes at 0.3-m and 0.75-m increased while those at 1.3-m became increasingly negative (Fig. 4.2.a). This reversal most likely arises from the combination of declining evapotranspiration, rising SWC and peak leaf fall. Following leaf fall there would be little active transport of soil water into the stem via evapotranspiration. There would, however, be a concentration gradient between relatively high soil CH_4 concentrations and lower atmospheric CH_4 concentrations. Diffusion of CH_4 through tree roots, up the stem and out into the atmosphere could represent a means for the system to reach chemical equilibrium hence the increase in stem CH_4 fluxes at 0.3-m and 0.75-m. At 1.3-m, atmospheric CH_4 likely exceeded stem concentrations which increased stem CH_4 uptake.

The important caveat surrounding tree stems and trace GHG exchange is that the mechanisms underlying stem exchange of CH_4 and N_2O are not fully understood. Whilst it is likely to be a predominantly passive process, there is a gap in our knowledge as to whether tree stems are "re-capturing" trace gases emitted from the soil or are taking up gases from the free atmosphere. Further, the results presented in this chapter demonstrate that fluxes of trace GHG can be positive or negative at the same sampling time depending on the height of the stem measurement. This could indicate that tree stems are "recycling" trace gases emitted lower down the stems and are therefore not actively removing trace GHGs from the atmosphere. This possibility could be tested using labelled litter or gases injected into the soils to try and trace the origins of gases emitted from tree stems.

4.5 Conclusion

This study presents a full seasonal record of CH_4 fluxes from tree stems of different species at multiple sampling heights in a temperate woodland on free-draining soils, as well as the first tree stem measurements of N_2O from such an ecosystem. The results indicate that tree stem CH_4 fluxes decline with stem sampling position above the forest floor, with mean fluxes becoming slightly negative at 1.3-m. Should this be generally representative of temperate woodlands and forests on free-draining soils, tree stem uptake of CH_4 above 1.3-m stem height could increase the size of the ecosystem CH_4 sink. Tree stem N_2O fluxes are highly variable but overall tree stems were sources of N_2O at both 0.3-m and 1.3-m sampling height. Therefore current models of temperate forest N_2O emissions may underestimate their contribution to the global terrestrial source. As temperate wooded ecosystems on free-draining soils represent the majority of the global forested area, their contribution to global trace GHG budgets needs to be investigated further.

Chapter 5: Tree stem CH_4 fluxes vary with sampling height in temperate and tropical forests on free-draining soils

Abstract

- **The majority of studies of tree stem methane (CH_4) fluxes have concentrated on wetland ecosystems but tree stems on free-draining upland soils can emit varying amounts of CH_4 at different heights.**
- **This study aimed to assess how stem CH_4 fluxes varied with sampling height in two distinct forest ecosystems and to investigate how tree species and litter inputs influence CH_4 fluxes in temperate and tropical forests on free-draining soils.**
- **CH_4 fluxes from the stems of two common tree species were measured at four sampling heights in a lowland tropical forest in Panama, Central America and in mixed deciduous woodland in Oxfordshire, UK. At the tropical site, stem CH_4 flux was measured within experimental litter addition, litter removal and control plots during a period of 10 days. At the temperate site, measurements were made in undisturbed plots during a period of four months.**
- **Mean CH_4 fluxes declined from positive values at 0.3-m and 0.75-m sampling height to negative values at 1.3-m and 2-m at both sites. The height at which CH_4 fluxes 'pivoted' from positive to negative differed between species at both the temperate and tropical sites.**
- **Should these results be representative, pivot point height is consistent across temperate and tropical forests on free-draining soils. This would suggest that globally the majority of tree stems in these ecosystems are CH_4 sinks, with the potential to significantly increase the largest terrestrial CH_4 sink.**

5.1 Introduction

Globally, hardwood forests are estimated to be a potential annual source of ~60 Tg methane (CH_4; Rice *et al.,* 2010) with the majority of these forests on upland or free-draining soils. Free-draining, aerated soils provide 5% of the 632 Tg y^{-1} global annual CH_4 sink (Kirschke *et al.*, 2013), however recently published data suggest that tree stem CH_4 fluxes in wetland forests may significantly increase ecosystem CH_4 emissions. If the same is true for forests on free-draining soils, this could potentially reduce the overall sink strength of these ecosystems.

As wetlands provide ideal conditions for methanogenesis, the majority of published studies have focused on temperate and tropical wetland trees. Tree stem CH_4 fluxes in tropical peat forests in Indonesia can account for 62-87% of total ecosystem CH_4 fluxes (Pangala *et al.* 2013). The

majority of CH_4 emitted from tree stems is not produced by the trees themselves but is produced in the soil by methanogenic consortia and transported into tree stems dissolved in soil water. Mesocosm studies of temperate tree and herbaceous plant species found that stem CH_4 emissions reflect soil CH_4 concentrations (Rusch and Rennenberg, 1998; Nisbet *et al.*, 2009), which has also been confirmed *in situ* for mature tree stems (Terazawa *et al.*, 2007). Several studies investigating CH_4 emissions from tree stems found that emissions declined with stem height, with the largest emissions measured closer to the soil surface (Pangala *et al.*, 2013; Pangala *et al.*, 2015; Wang *et al.*, 2016). Inter-species differences in physiology can affect gas transport and exchange through tree stems; in a tropical peat forest stem CH_4 fluxes were negatively correlated with wood specific density (greater density slows diffusion) and positively related to stem lenticel density as there are more pores for gas exchange to occur through (Pangala *et al.*, 2013). Consequently, the variation of CH_4 fluxes with stem height could also differ among species.

In aerated free-draining soils, the concentrations of CH_4 are much lower compared to wetland soils, and therefore tree stems are also likely to be a smaller source of CH_4. Indeed, if the same pattern of declining methane fluxes with stem height is observed for trees on free-draining soils, they could potentially represent a CH_4 sink. Trees do not oxidise CH_4 directly, however they would provide a conduit for CH_4 to be transported into the surrounding soil along with atmospheric oxygen where it would be consumed by methanotrophic consortia. Presently there is no evidence of tree stem uptake of CH_4, however fluxes of 0 $\mu g\ m^{-2}\ h^{-1}$ were recorded at a sampling height of 1.3-m in a Chinese upland forest (Wang *et al.*, 2016). This suggests that tree stem uptake of CH_4 may be possible higher up tree stems when abiotic and biotic factors favour CH_4 oxidation in the soil.

This chapter describes a study of variation in tree stem CH_4 fluxes with sampling height in a temperate woodland and a tropical forest on free-draining soils, which aimed to test the following hypotheses:

H1) Tree stem CH_4 fluxes will be greatest at the bottom of tree stems and decline with sampling height, leading to uptake of CH_4 further up tree stems.

H2) The height at which CH_4 fluxes change from emission to uptake (the pivot point) will vary between species because of physiological differences, such as wood specific density, which affect the diffusion of CH_4 from xylem tissue to the atmosphere.

5.2 Materials and Methods

5.2.1 Field areas and sampling

5.2.1a Gigante Peninsula, Panama, Central America

Measurements in the tropical forest were carried out between 18th November and 27th November 2015 within the five control plots of the Gigante Litter Manipulation Project (GLiMP)

approximately 5 km south of Barro Colorado Island (BCI), Panama, Central America. The plots were set up between 2000 and 2002; each plot measures 45-m × 45-m and the edges of the plots were trenched to a depth of 0.5-m, lined with plastic and then backfilled. A full description of the litter manipulation experiment is given in Sayer *et al.* (2006) and Sayer and Tanner (2010). The mean annual temperature at the weather station on BCI is 26ºC, mean annual rainfall is 2,600 mm and there is a strong dry season from mid-December to mid-April (Leigh, 1999).

Two common tree species were selected for this study: the fast-growing canopy tree *Simarouba amara* (Aubl.) and the shade-tolerant subcanopy tree *Heisteria concinna* (Standl.). Both species have relatively smooth bark and straight stems, which facilitates sampling. Tree stem gas fluxes were measured using a flexible chamber made from a 450-mm × 300-mm sheet of polycarbonate (Bay Plastics Ltd, North Shields, UK), lined with neoprene foam (19 mm wide, 25 mm thick; Seals+Direct Ltd, New Milton, UK). The chambers were secured to the tree stems at 0.3-m, 0.75-m, 1.3-m and 2-m height using cam buckle straps. Gas samples were taken by syringe from a septum in the middle of the chamber at 0, 5, 10 and 15 minutes, and injected into pre-evacuated 12-ml borosilicate vials (Exetainer™, LabCo Ltd, High Wycombe, UK). Air pressure and temperature outside the stem chamber were recorded at the start of sampling using a Commeter C4141 Thermometer-Hygrometer-Barometer probe (Comet Systems, Czech Republic). Soil temperature at 0-10-cm depth was measured adjacent to the trees using a soil temperature probe. Daily total solar radiation was calculated from daytime measurements made at 15-minute intervals on the meteorological tower on BCI at 48-m height using a LiCor LI200X pyranometer (LiCor, Nebraska, USA; data provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute).

5.2.1b Wytham Woods, Oxfordshire, UK

Measurements at the temperate site were conducted in the control plots of an existing litter manipulation experiment at Wytham Woods, an old growth (~120-years) mixed deciduous woodland in Oxfordshire, UK. The canopy at the study site is dominated by Ash (*Fraxinus excelsior* L.), Beech (*Fagus sylvatica* L.), Sycamore (*Acer pseudoplatanus* L.) and Oak (*Quercus robur* L.*;* Fenn *et al.* 2014). In summer 2013, 15 experimental plots measuring 25-m × 25-m each, were established in five replicate blocks. Each plot was trenched to a depth of 0.5-m, one wall was lined with plastic to limit the transfer of water and nutrients by root and hyphal networks, and the trenches were then backfilled. Within each plot, four soil collars (200-mm internal diameter and 120-mm height) were embedded into the soil to 30-mm depth. The collars were installed *c*. 7.5-m from the centre of each side of the plots in July 2013. Full details of the experimental design are given in Lopez-Sangil *et al.* (2017) and Chapter 4 of this thesis. Only four of the five control plots were used in this study, as one of the control plots did not contain any Sycamore trees. In four of

the five control plots, three individuals each of Ash and Sycamore were randomly selected, making a total of 24 trees. All trees were marked at 1.3-m and the girth was measured at 0.3, 0.75, 1.3, and 2-m. Tree stem CH_4 fluxes were sampled using the same chamber design, sampling heights, and procedure as described above but Play-Doh (Hasbro, United Kingdom) was used to seal fissures and bind the chamber the tree bark (Chapter 4) and gas samples were collected at 0, 3, 6 and 10 minutes.

Air pressure and temperature outside the stem chamber were recorded at the start of sampling using a Commeter C4141 Thermometer-Hygrometer-Barometer probe (Comet Systems, Czech Republic) and soil temperature at a 6-cm depth was recorded adjacent to the trees using a Thermapen (ETI Ltd, Worthing, UK). Volumetric soil water content (SWC) at a depth of 0-6-cm depth was measured monthly using a Thetaprobe (Delta-T Devices, Cambridge, UK) calibrated to local soil conditions following the manufacturer's instructions. Data for monthly mean solar radiation and total rainfall were collected at the weather station in Wytham Woods (UK Environmental Change Network). All samples were analysed at the Open University, UK using off-axis Integrated Cavity Output Spectroscopy (FMA-200 Fast Methane Analyser; Los Gatos Research, Mountain View, CA, USA).

5.2.2. Data analyses

Greenhouse gas flux data often features a small number of extreme outliers; although these values are not necessarily due to measurement error, they are often the result of biological activity (Megonigal *et al.*, 2008; Covey *et al.*, 2012), which could obscure patterns due to tree species identity or abiotic factors. Consequently, the data were inspected visually and extreme outliers that lay outside of the 5^{th} - 95^{th} interquartile range were removed. As a result, 4 of the 385 CH_4 flux values were removed from the temperate data and 4 of 153 CH_4 flux values were removed from the tropical data. All statistical analyses of CH_4 stem fluxes and pivot point heights were conducted with and without outliers and full results of the analyses including litter treatments (for the Panama CH_4 flux data) and extreme outlier values are included in Appendix IV.

All data analyses were conducted in R 3.3.2 (R Core Team, 2016) using the lme4 package for mixed effects models (Bates *et al.*, 2015). CH_4 fluxes were calculated for each chamber following Baird *et al.* (2010), whereby the least squares linear regression slope of the four sample concentrations is plotted against sampling time and the slope to give the CH_4 flux in $\mu g\ m^{-2}\ h^{-1}$. CH_4 flux measurements were only used for further statistical analysis if the R^2 of the regression was >0.7; this cut-off point was chosen following Alm *et al.* (2007; cited in Cooper *et al.*, 2014), who noted that low fluxes (especially those near to zero) tend to have low R^2 values. Effects of sampling height on stem CH_4 fluxes were assessed using linear mixed effects models (*lmer* function), with sampling height as a fixed effect, and plot and time as random effects. Once a significant height

effect was identified, pivot points (i.e. the height at which stem fluxes are 0 μg m^{-2} h^{-1}) were calculated from least squares linear regression of the four stem flux values per tree per sampling date plotted against sampling height, whereby the pivot point is equal to the negative of the y-intercept divided by the gradient of the regression slope.

Effects of climatic variables on stem pivot points were assessed using linear mixed effects models (*lmer* function), with soil temperature, precipitation, solar radiation and their interaction as fixed effects, and plot and time as random effects. The significance of each term was determined by comparing nested models using likelihood ratio tests. Models were simplified by sequentially dropping terms until a minimum adequate model was reached, using AICs and *p*-values to check for model improvement (Pinheiro and Bates 2000). Having identified which climatic variables to include as covariates, tree species was included as a fixed effect and the models were then compared as above to arrive at a new minimum adequate model. Effects of seasonal variation were tested by comparing minimum adequate models with and without time as a random effect. The final model fit was inspected using diagnostic plots. Statistics for mixed effects models are given for the comparison between the best-fit model and the corresponding null model. All results are reported as significant at $p < 0.05$ but, due to the low number of replicate plots ($n = 4$), marginally significant trends are also reported at $p < 0.1$.

5.2.3. Upscaling models

Stem fluxes were estimated for sections of tree stems at Wytham Woods to 2.5-m and 15-m height (the average canopy height at Wytham Woods is 15-18-m; Herbst *et al.*, 2007), using the linear regression equations obtained from plotting CH_4 fluxes against height for each species and plot. As CH_4 fluxes were not sampled at 2-m stem height until October 2015, CH_4 fluxes at 2-m for each species and plot for the preceding months (February 2015 to September 2015) were estimated from the relationship between fluxes and height during October 2015 to January 2016. CH_4 fluxes from 2.5 to 15 m stem height were assumed to be equal to the fluxes measured at 2.2 - 2.5 m as there presently exists no published data to refute the assumption. The surface area for unsampled stem sections was estimated from stem diameter measurements at each sampling height and stem CH_4 fluxes at each height were multiplied by the corresponding surface area. All calculations were carried out for individual trees, and these data were subsequently used to estimate the mean CH_4 flux per tree for the ecosystem (given as g ha^{-1} d^{-1}). The mean CH_4 flux per tree was then multiplied by the number of trees per hectare to obtain total tree emissions per unit area. To estimate dry season fluxes in Panama, the available stem CH_4 fluxes collected during the 2014 campaign (Chapter 3) were entered into the models of stem fluxes based on the data from the 2015 campaign (this chapter). Monthly estimated CH_4 fluxes up to 2.5-m and 15-m stem height at Wytham Woods are presented in Appendix VI.

Soil CH_4 fluxes per hectare (given as g ha^{-1} d^{-1}) were estimated by subtracting total tree basal area from a square hectare and multiplying measured soil CH_4 fluxes with this surface area.

Seasonal estimates for CH_4 fluxes in Panama assumed a dry season length of 120 days and a wet season length of 245 days (for 2014/15 the dry season was 143 days and wet season 195 days likely influenced by the El Nino that year); the daily mean CH_4 fluxes from the three litter treatments were multiplied by the number of days and summed to give seasonal totals across all treatments. Annual total ecosystem CH_4 flux contributions at Wytham Woods were estimated by calculating monthly totals from the daily values and then summing the monthly totals to give an annual value.

5.3 Results

5.3.1 Variation in stem CH_4 fluxes with sampling height

There was significant variation in stem CH_4 fluxes with sampling height at the tropical ($p < 0.0001$, $r^2 = 0.235$, $\chi 2 = 38.7$; Fig. 5.1) and temperate site ($p < 0.0001$, $r^2 = 0.235$, $\chi^2 = 97.9$; Fig. 5.2) and the patterns were similar between sites, whereby CH_4 fluxes were largely positive at 0.3-m and declined with sampling height, becoming mostly negative at 2-m stem height.

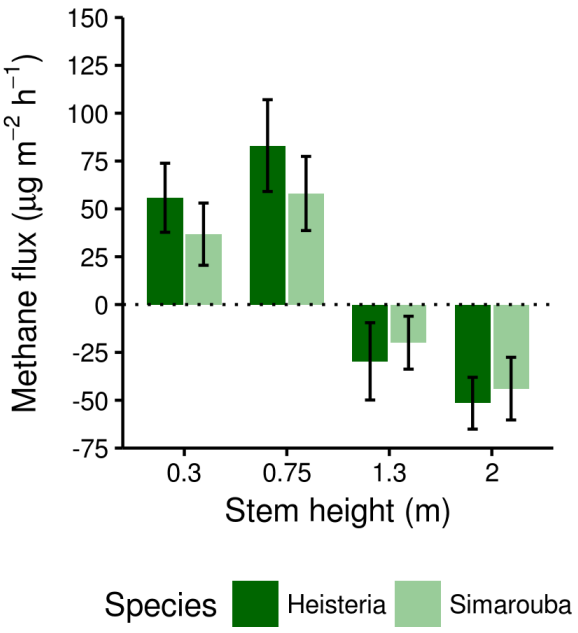

Figure 5.1 Bar plot of mean methane (CH_4) fluxes against sampling height measured from stems of two common tree species: *Heisteria concinna* (dark green) and *Simarouba amara* (light green) in experimental litter manipulation treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015; error bars show the standard error of means for $n = 4$. Tree stem CH_4 fluxes are pooled from all litter manipulation treatments as no significant treatment effects were observed.

At both sites, mean stem CH_4 fluxes were significantly lower from the fast-growing species, *Simarouba* ($p < 0.01$, $r^2 = 0.303$, $\chi^2 = 10.5$) and Ash ($p < 0.05$, $r^2 = 0.235$, $\chi^2 = 5.47$), respectively, compared to *Heisteria* and Sycamore.

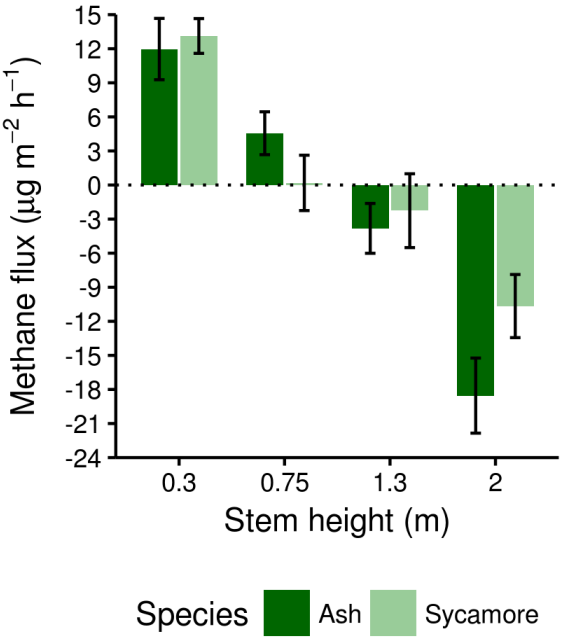

Figure 5.2 Bar plot of mean methane (CH_4) fluxes against sampling height measured from stems of two common tree species: Ash (dark green) and Sycamore amara (light green) in a temperate deciduous woodland on free-draining soil in Oxfordshire, UK, between October 2015 and January 2016; error bars show the standard error means for $n = 4$. Means are based on four replicates per species.

5.3.2 Controls of variation in stem CH_4 fluxes with sampling height

At both sites, climatic variables affected stem CH_4 fluxes but the influence varied with sampling height. Between 18th November 2015 and 17th November 2015 in Panama, air temperature measured in the plots ranged from 24-28.3°C (mean: 25.8°C), soil temperature ranged from 25.8 -29.3°C (mean: 26.4°C), daily mean solar radiation ranged from 11 – 218 Wm^{-2} (mean: 161 Wm^{-2}) and total daily precipitation ranged from 0 – 21.3 mm (mean: 7.61 mm). During the sampling period at Wytham Woods, air temperature measured in the plots ranged from 4.6 – 15.8 °C (annual mean: 13.3°C), soil temperature ranged from 8.6 -14.2°C (annual mean: 11.9°C), and soil water content ranged from 45.3 – 69.8% (annual mean: 44%).

At 0.3-m, CH_4 fluxes increased with solar radiation in Panama ($p < 0.05$, $r^2 = 0.354$, $\chi^2 = 5.94$) and with soil temperature at Wytham ($p < 0.05$, $r^2 = 0.043$, $\chi^2 = 3.81$). At 0.75-m height, CH_4 fluxes were not significantly affected by abiotic factors in Panama or Wytham. At 1.3-m, stem CH_4 fluxes were highest on sampling days with high rainfall Panama ($p < 0.1$, $r^2 = 0.151$, $\chi^2 = 2.77$) and were significantly related to soil temperature at Wytham ($p < 0.01$, $r^2 = 0.095$, $\chi^2 = 7.45$; soil temperature × rainfall interaction: $p < 0.05$, $r^2 = 0.097$, $\chi^2 = 7.56$). Finally, at 2-m sampling height,

there were no relationships between stem CH_4 fluxes and abiotic factors at either the tropical or temperate site.

5.3.3 Pivot Points

At both sites, the height of the pivot point at which stem CH_4 fluxes switched from emission to uptake was *c.* 1.3-m. In Panama, median stem CH_4 fluxes at 1.3-m height were -3.75 µg CH_4 m^{-2} hr^{-1} and -25.8 µg CH_4 m^{-2} hr^{-1} for *Heisteria* and *Simarouba,* respectively. At Wytham Woods, median stem CH_4 fluxes at 1.3-m were -8.96 µg CH_4 m^{-2} hr^{-1} for Ash and 2.50 µg CH_4 m^{-2} hr^{-1} for Sycamore.

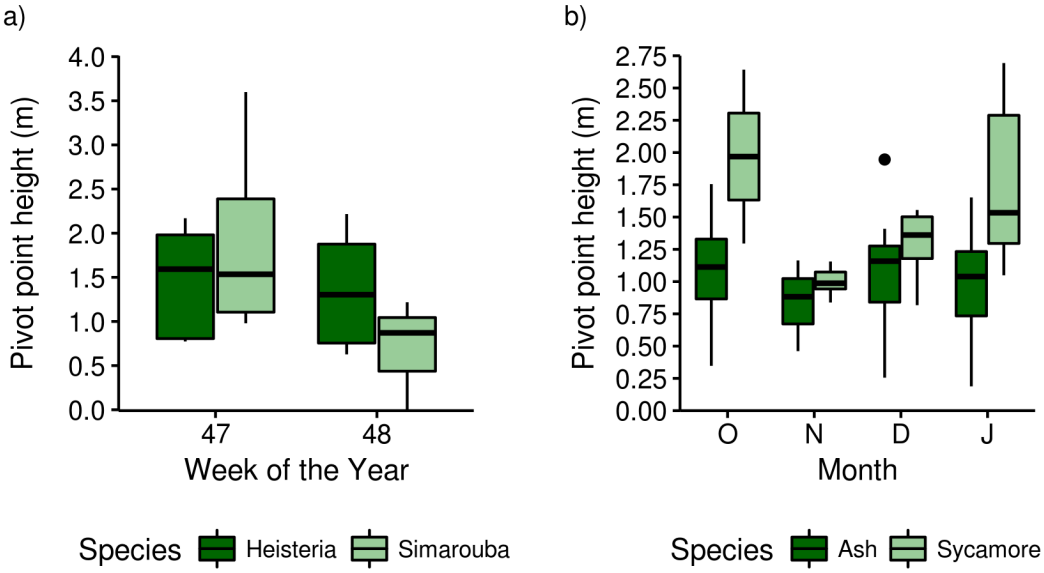

Figure 5.3 Ranges of pivot points from a) two common tropical tree species: *Heisteria concinna* (dark green) and *Simarouba amara* (light green) in the control plots of the Gigante Litter Manipulation Plots (GLiMP) in a lowland tropical forest on free-draining soil in Panama, Central America between 18th November and 27th November 2015; b) two common temperate forest species: Ash and Sycamore on free-draining soils in Oxfordshire, UK, between October 2015 and January 2016. Ranges are based on four replicates per species.

There were also significant differences in pivot points between species at both sites. In Panama, pivot point heights were generally higher for *Heisteria* stems than *Simarouba* stems ($p < 0.001$, $r^2 = 0.998$, $\chi^2 = 14.2$; Fig. 5.3a). At Wytham Woods, the pivot points for Ash stems were lower than for Sycamore during all four months of sampling ($p < 0.01$, $r^2 = 0.300$, $\chi^2 = 8.45$; Fig. 5.3b). At both sites pivot point heights were positively related to moisture. In Panama, pivot point height was marginally higher on days with elevated rainfall ($p < 0.1$, $r^2 = 0.950$, $\chi^2 = 2.95$). Pivot point height at Wytham Woods was greater when soils were warmer and wetter (soil temperature × SWC interaction: $p < 0.1$, $r^2 = 0.153$, $\chi^2 = 7.15$).

5.3.4 Regional and global results

As tree stem CH_4 fluxes in the tropical forest were only measured at four heights during two weeks at the end of the 2015 wet season, the estimated ecosystem contributions should be interpreted with caution. It was assumed that the CH_4 fluxes sampled in November 2015 were broadly representative of wet season tree stem and soil CH_4 fluxes.

As expected, the soil was a CH_4 sink during the dry season, and although the lower 2.5-m portion of tree stems was estimated to be a source of CH_4 (with the exception of tree stems in the litter addition plots), the ecosystem-level tree stem CH_4 sink increased when CH_4 fluxes were modelled up to 15-m stem height (Table 5.1).

Litter Treatment	**Dry Season Flux (g ha^{-1} d^{-1})**			**Wet Season Flux (g ha^{-1} d^{-1})**		
	Tree 2.5-m	Tree 15-m	Soil	Tree 2.5-m	Tree 15-m	Soil
L-	0.75±1.56	-11.3±12	-12.2±6.81	2.06±3.44	-3.88±6.33	2.9±4.38
CT	1.08±1.63	-8.5±10	-13.6±8.91	0.53±0.47	-3.83±4.72	4.67±4.16
L+	-0.86±3.17	-13.2±6.94	-7.42±8.08	0.68±3.31	-4.96±4.43	7±4.12
Mean	0.33±2.12	-11±9.65	-11.1±7.93	1.09±2.41	-4.22±5.16	4.86±4.22

Table 5.1 Table of estimated daily ecosystem CH_4 flux contributions from tree stems up to 2.5-m stem height, 15-m stem height and the soil surface in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, during the dry (mid-December to late April) and wet (late April to mid-December) seasons. Tree stem CH_4 flux estimations were based on pooled CH_4 flux values from *Heisteria concinna* and *Simarouba amara*. All CH_4 fluxes were pooled across litter manipulation treatments prior to upscaling.

The soil in all three litter treatments became a source of CH_4 during the wet season, with the lowest estimated contributions from litter removal plots and the greatest from litter addition plots. Tree stem CH_4 fluxes during the wet season showed a similar pattern to the dry season: the lower 2.5-m portion of the stems was a source of CH_4 but the trees became sinks for CH_4 when the model included up to 15-m stem height. Stem uptake of CH_4 offset soil CH_4 emissions considerably and trees stems in the litter removal plots compensated completely for soil CH_4 emissions.

The seasonal estimates show that tree stems up to 2.5-m constitute a source of 307 g CH_4 ha^{-1}, whereas tree stems up to 15-m provide net sink of -2354 g CH_4 ha^{-1} and the soil a sink of -141 g CH_4 ha^{-1}. Assuming that tree stem flux estimates up to 15-m are representative of whole tree stem CH_4 fluxes, then the forest on the Gigante peninsula could be a CH_4 sink of -2495 g ha^{-1} over the course of a year.

Season	Total Seasonal Flux (g ha^{-1})		
	Tree (2.5-m)	Tree (15-m)	Soil
Dry	39.6±254	-1320±1158	-1332±952
Wet	267±591	-1034±1264	1191±1034

Table 5.2 Table of estimated seasonal ecosystem CH_4 flux contributions from tree stems up to 2.5-m stem height, 15-m stem height and soil surfaces in a lowland tropical forest on free-draining soil in Panama, Central America, during the dry (mid-December to late April) and wet (late April to mid-December) seasons. Tree stem CH_4 flux estimates were based on combined values from both *Heisteria concinna* and *Simarouba amara* and all CH_4 flux values were pooled from all the litter manipulation plots prior to upscaling.

In contrast to Panama, Wytham Woods was a CH_4 source for most of the year (Fig. 5.4). The majority of tree stem emissions occurred in the spring and summer, whereas tree stems were a net sink in the autumn and winter. Tree-mediated CH_4 fluxes (based upon the lowest 2.5-m of stems) varied from 0.54 ±0.24 g ha^{-1} d^{-1} in spring to -0.29 ±0.16 g ha^{-1} d^{-1} in autumn. When estimates were made assuming a tree height of 15-m, tree-mediated fluxes ranged from 23.14 ±10.14 g ha^{-1} d^{-1} in spring 2015 to a sink of -12.64 ±6.81 g ha^{-1} d^{-1} in autumn 2015. Consequently, the ecosystem CH_4 sink was significantly enhanced between September and November 2015 when both tree stems and soil were CH_4 sinks.

Annual total ecosystem CH_4 fluxes were 65.1 g ha^{-1} yr^{-1} from tree stems up to 2.5-m height, 2798 g ha^{-1} yr^{-1} up to 15-m stem height and 579 g ha^{-1} yr^{-1} from soils, making Wytham Woods a potential net source of CH_4. If modelling assumptions are correct and the estimated stem flux for 15-m height is representative of whole tree CH_4 fluxes, then the combined CH_4 emission from trees and soil at Wytham Woods would be 3377 g CH_4 ha^{-1} yr^{-1}.

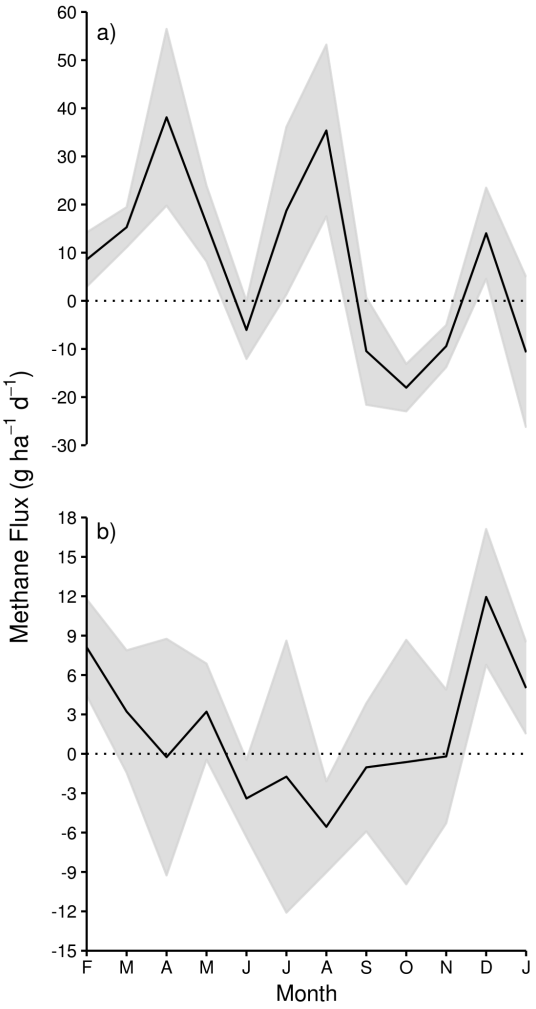

Figure 5.4 Seasonal patterns of methane (CH_4) fluxes in a temperate woodland on free-draining soil in Oxfordshire, UK, showing estimated monthly contributions to ecosystem CH_4 fluxes from a) tree stems up to 15-m height based on combined fluxes from both ash and sycamore stems and b) soil surfaces between February 2015 and January 2016. Grey shading marks the standard deviation.

5.4 Discussion

These results show for the first time a persistent pattern in the transition of stem CH_4 fluxes from positive to negative with sampling height in temperate woodland and tropical forest trees. Previous studies in the Amazon (Pangala *et al.*, 2013), China (Wang *et al.*, 2016) and the USA (Megonigal *et al.*, 2016) have all reported decreasing CH_4 fluxes with increasing sampling height across multiple species.

Stem lenticel density, wood specific density and stem diameter can all influence stem CH_4 fluxes. Stem lenticels are structures that aid gas exchange and are an important adaptive-structure in flood tolerant species (Kozlowski, 1997; Rusch and Rennenberg, 1998; Purjava *et al.*, 2004). However, given that the forest at both sites grows on free-draining soils, it is unlikely that adaptive changes in stem lenticels resulted in significant effects on stem CH_4 fluxes. Specific wood density influences diffusion rates through the tree stem: as wood density increases, the diffusion of stem

CH_4 to the atmosphere declines. The influence of stem diameter on CH_4 fluxes is likely a function of wood density because woody tissue gets denser with age (Chave *et al.*, 2009), and stem diameter increases with tree age (Stephenson *et al.*, 2014), so older trees emit less CH_4 than younger, faster-growing trees of the same species (Pangala *et al.*, 2015). Wood specific density and stem diameter were both negatively correlated with tree stem CH_4 fluxes in a tropical peatland forest (Pangala *et al.*, 2013) and temperate wetland species (Rusch and Rennenberg, 1998; Terazawa *et al.*, 2007). However, stem CH_4 fluxes in Panama were greater from *Heisteria* stems, which have a greater wood specific density than *Simarouba* (Condit *et al.*, 2013). The difference between the wood specific densities of Ash (0.53 g cm^{-3}) and Sycamore (0.49 g cm^{-3}; Wiemann *et al.*, 2007) is minor and is therefore also unlikely to explain the differences in stem CH_4 fluxes between species. The lack of a clear relationship between stem density and CH_4 fluxes, suggests that in forests on free-draining soils, wood specific density is not an important control of variation in tree stem CH_4 fluxes.

Abiotic factors were significant controls of CH_4 fluxes and pivot point heights at both sites. In Panama, stem CH_4 fluxes at 0.3-m sampling height were greater on sampling days with higher amounts of solar radiation, whereas pivot point height was significantly higher on wetter sampling days. Rates of photosynthesis in the canopy increase with solar radiation, leading to increased transport of water from the soil into trees. As the majority of tree-mediated CH_4 emissions originate in the soil (Terazawa *et al.*, 2007), enhanced rates of water transport could increase stem CH_4 fluxes lower on tree stems as more CH_4 is transported out of the soil. However in Panama pivot point heights were generally greater for the subcanopy species *Heisteria*. This suggests that although pivot point height could increase with solar radiation, changes in soil CH_4 production as a consequence of soil wetting may be more important. At Wytham Woods, pivot point height was greatest when soils were warmer and wetter. Although solar radiation was also shown to significantly increase CH_4 fluxes at 0.3-m and 1.3-m stem height during the growing season (Chapter 4), the majority of measurements for this study were taken after leaf abscission and therefore the effects of solar radiation on pivot point height at Wytham were not considered.

The similarities between sites in the relationship between soil water content and pivot point height lends further support to the hypothesis that changes in soil CH_4 production and consumption are an important control of variation in stem CH_4 fluxes with height. Greater concentrations of CH_4 in water taken up by trees is a plausible mechanism for the greater pivot point heights observed during wet periods. As SWC increases, soil oxygen concentrations decline, creating favourable conditions for methanogenesis (Borken *et al.*, 2006). As CH_4 concentrations in the soil rise, more CH_4 is dissolved in soil water and transported up the tree stems.

It is striking that mean pivot point heights were similar between sites (1.31 ±0.15-m in Wytham and 1.44 ±0.19-m in Panama) and among species. Whereas very weak CH_4 uptake (< -0.07 μmol m^{-2} h^{-1}) was recorded at 0.3 - 0.6-m stem height in an upland forest in the USA (Pitz and

Megonigal, 2017), CH_4 fluxes of 0 μg CH_4 m^{-2} hr^{-1} were found at 1.3-m sampling height from Poplar stems in an upland forest in China (Wang *et al.*, 2016) This suggests that there may be a global pattern of stem CH_4 uptake above certain stem heights in forests on free-draining soils. However, it is unclear how much of the CH_4 uptake by trees in forests on free-draining soils is from the free atmosphere. It is possible that negative CH_4 stem fluxes observed at 1.3-m and 2-m stem height are "recycling" of CH_4 emitted lower down the stem in a repeating pattern of emission and uptake along the stem. In this case, tree CH_4 budgets would be neutral.

Biotic controls arising from the interaction between trees and soils could also be an important control of variability in stem CH_4 fluxes with tree height. As discussed in Chapter 4, rates of CH_4 uptake increase with fine root density (Fender *et al.*, 2013) and litter quality can also affect methanogenesis and methanotrophy (Aubert *et al.*, 2010). Soil CH_4 concentrations in both temperate and tropical free-draining soils have been shown to decrease with soil depth as the soil transitions from the organic to mineral layers (Adamsen *et al.*, 1993; Koehler *et al.*, 2012). Faster growing tree species tend to have deeper maximum root depths than slower growing species and so are more likely to be drawing water from soil depths that have lower concentrations of dissolved CH_4 (Koehler *et al.*, 2009; Chapter 3). This may explain why the highest pivot point calculated in the present study was for an Ash tree, because lower concentrations of CH_4 being transported up the stem would result in a weaker diffusion gradient between the stem interior and the free atmosphere. This outlier pivot point height was omitted from analyses as it was several metres higher than the next highest pivot point at Wytham, suggesting that it could be a measurement error. However, correct identification of pivot point heights in both temperate and tropical forests is important because higher pivot points could substantially alter global estimates of total stem CH_4 emission and uptake by tree stems globally. As a result stem CH_4 fluxes at all heights will be lower as less CH_4 can be transported from the soil into the trees. Stem CH_4 fluxes were lower from the faster growing species at each site, which were more likely to be sourcing water from deeper in the soil profile, which has lower dissolved CH_4 concentrations. Consequently, the tree stem internal CH_4 concentration would reach equilibrium at a lower height (i.e. the pivot point decreases) as less CH_4 is transported into the trees. However given that Sycamore grows almost as fast as Ash and *Simarouba* had a greater pivot point than *Heisteria*, to establish the veracity of this relationship more species will need to be sampled in tropical and temperate forests.

5.5 Conclusion

These results show that in temperate and tropical forests on free-draining soils, tree stem fluxes pivot from positive fluxes below 1-m stem height to negative fluxes above 1.3-m. Consequently, tree stems may not be the net source of CH_4 that recent research has indicated; the majority of mature forest trees grow well in excess of two metres stem height and therefore stem uptake may be the dominant process over the majority of tree stem surface area. Pivot point height and its controlling factors (e.g. climate, species and root-soil interactions) need to be better understood as pivot point height will determine the stem surface area that CH_4 uptake takes place on. Further should the findings from Panama and Wytham be representative globally then whilst tropical lowland forests on free-draining soils may be CH_4 sinks, temperate lowland forests on free-draining soils could negate the CH_4 sink commonly attributed to those areas.

Chapter 6 – Discussion and Synthesis

6.1 Introduction

The research presented in this thesis explored whether forests on free-draining soils in temperate and tropical regions were sinks or sources of methane (CH_4) and nitrous oxide (N_2O) and how the fluxes of trace greenhouse gases (GHG) from these forests varied seasonally. In this chapter, the implications of the research will be discussed through the lens of the hypotheses outlined in Chapter 1. The findings of the studies will be synthesised and recommendations for further work will also be set out.

This body of work demonstrates that:

- Tree stem CH_4 and N_2O fluxes did not vary seasonally in a tropical rainforest, however there were weak seasonal changes in tree stem CH_4 and N_2O fluxes in a temperate woodland.
- Tree stems in temperate and tropical forests on free-draining soils emitted CH_4 and N_2O at the base. Stem CH_4 and N_2O fluxes decreased with sampling height to mostly uptake at >1.3-m stem sampling position.
- Stem trace GHG fluxes varied significantly between species at both the tropical and temperate forest sites.
- Tree stem and soil CH_4 and N_2O fluxes in a tropical rainforest were influenced by changes in litter quantity.

6.2 Hypotheses revisited

6.2.1 Seasonal variation of tree stem and soil CH_4 and N_2O fluxes

Hypothesis one was evaluated at a lowland tropical rainforest site in Panama (Chapter 3) and in a temperate woodland in the UK (Chapter 4). Tree stem fluxes of CH_4 and N_2O at 0.3-m sampling height in Panama did not show significant temporal variation over the dry to wet season transition in 2014. The lack of seasonal trends in tree stem CH_4 and N_2O fluxes in Panama was surprising, as it was hypothesized that seasonal changes in soil water content (SWC) in free-draining soils would enhance soil methanogenesis and denitrification, which in turn would result in higher tree stem trace GHG fluxes during the wet season. Soil CH_4 fluxes increased significantly from uptake in the dry season to emission in the wet season in Panama (Fig. 3.2) but no significant change was observed in soil N_2O. A study of nitrogen fertilization effects in the same area of tropical forest also reported significant seasonal variation in soil N_2O, with higher N_2O fluxes in the wet season than the dry season fluxes (Koehler *et al.*, 2012). The lack of a seasonal response in stem CH_4 fluxes, despite a significant change in soil CH_4 fluxes, suggests that biotic factors such as root-soil

interactions and microbial community composition may be affecting tree-mediated fluxes of trace GHGs obscuring seasonal trends. At Wytham Woods, tree stem CH_4 fluxes at 0.3-m and 1.3-m stem height showed a trend towards temporal changes during sampling. Mean CH_4 fluxes at 0.3-m were lowest in July 2015 and peaked in December 2015, and at 1.3-m decreased significantly throughout sampling (Fig. 4.2). A similar pattern of non-significant seasonal changes in stem and soil CH_4 was found in upland catchment forest in Maryland, USA, where stem fluxes were largely positive throughout sampling and soil CH_4 fluxes were lowest in July (Warner *et al.*, 2017). No temporal variation was found at Wytham Woods in soil CH_4 and N_2O, however fluxes of both were lowest during the summer of 2015 (Fig. 4.2 & 4.6). Whilst summer months in temperate zones tend to be drier than winter months there is not the same disparity in precipitation and soil moisture seen in the tropics leading to non-significant seasonal patterns of CH_4 and N_2O in these ecosystems.

Tree stem CH_4 and N_2O fluxes were not significantly related to increased air temperature, soil temperature or SWC in Panama. However, soil CH_4 fluxes increased as rainfall rose and air temperature declined over the dry-to-wet season transition (Fig. 3.2). Soil N_2O fluxes were positively correlated with rising SWC, consistent with responses in the Amazon (Liengaard *et al.*, 2014) and Costa Rica (Hall *et al.*, 2013). The lack of variation in tree stem and soil CH_4 and N_2O fluxes is most likely due to the narrow range of temperatures recorded during sampling in Panama (Fig. 3.1). As changes in air temperature, soil temperature and SWC were greater at Wytham Woods, this may explain the greater influence of abiotic factors on tree stem and soil trace GHG fluxes in the temperate woodland compared to the tropical forest. As soil temperatures rose tree stem CH_4 fluxes at 0.75-m decreased slightly (Fig. 4.3.a). Tree stem N_2O fluxes at 0.3-m increased with soil temperature (Fig. 4.7.a). This is consistent with the positive correlation between soil N_2O fluxes and soil temperature across European forests on free-draining soils (Barnard *et al.*, 2005; Gundersen *et al.*, 2012). The observation that soil CH_4 fluxes at Wytham increased with soil temperature and SWC shows that methanogenesis and methanotrophy at Wytham Woods respond in a typical manner to climatic variation. The lack of significant variation in stem CH_4 with air temperature, soil temperature and soil moisture observed at both sites is consistent with recently published data. A study of seasonal change in an upland forest found no significant relationship between air temperature and stem CH_4 fluxes (Wang *et al.*, 2016). At two temperate upland forests in the north-eastern USA, no significant relationships were found between tree stem CH_4 fluxes and air temperature, soil temperature or soil moisture (Pitz and Megonigal, 2017; Warner *et al.*, 2017). The observation of a small increase in stem N_2O fluxes at 0.3-m in Wytham Woods with soil temperature at 6-cm mirrors rising stem N_2O fluxes from black alder mesocosms and soil temperature at 8-cm depth (Machacova *et al.*, 2013). The most likely explanation of the apparent lack of temperature and moisture responses of stem CH_4 fluxes compared to soil CH_4 fluxes is that as tree stems are predominantly conduits of CH_4, there is a disconnect between methanogenesis and methanotrophy not present in measurements of soil CH_4 fluxes. This means that on some level, tree

stem fluxes of trace GHG will be regulated by physiology and metabolism in addition to external abiotic factors.

The interaction between tree stem trace GHG fluxes, tree species and abiotic factors is perhaps most revealing with regards to the effects of solar radiation. This study is the first to link changes in tree stem CH_4 and N_2O fluxes with solar radiation. Photosynthesis is affected by light availability and therefore, stomatal opening and subsequent water loss from the canopy provide a plausible mechanism linking solar radiation and stem CH_4 fluxes at Wytham. As the rate of photosynthesis increases, trees need to transport more water (Chaves *et al.*, 2002), which could increase transport of CH_4 and N_2O dissolved in soil water into tree stems.

At Wytham Woods, solar radiation had minor effects on tree stem CH_4 fluxes. 0.3-m CH_4 stem fluxes declined under higher solar radiation and 1.3-m stem fluxes were increased with solar radiation (Fig. 4.2); the differences with sampling height were unexpected, as it was assumed that the link between CH_4 fluxes and solar radiation would be the same at all heights. NPP, which is stimulated by solar radiation, has been hypothesised to be the cause of apparent diurnal cycling in CH_4 fluxes from temperate forest soils (Shoemaker *et al.*, 2014) and to generate 0.2-1 Tg CH_4 y^{-1} of foliar CH_4 emissions (Bloom *et al.*, 2010). To date there is no evidence of significant diurnal cycling of tree stem CH_4 fluxes however two studies noted that CH_4 fluxes peak in the late afternoon or at night (Wang *et al.*, 2016; Pitz and Megonigal, 2017). This peak stem efflux to later in the day could arise from a lag between an increase in root exudates during times of peak NPP and how quickly methanogens can metabolise the exudates.

Rates of water transport up tree stems increase with tree height (Goldstein *et al.*, 1998) so it was expected that stem fluxes of CH_4 and N_2O would be highest from canopy species. Unexpectedly, CH_4 fluxes were greater from shade-tolerant sub-canopy *Heisteria* stems than the fast-growing canopy species *Simarouba* but stem N_2O fluxes at 0.3-m were lower from *Heisteria* compared to *Simarouba* trees. Dissolved CH_4 concentrations in soils on the Gigante peninsula were found to decrease with depth and N_2O concentrations rose (Koehler *et al.*, 2009). As faster growing trees have deeper maximum root depths, this could explain the observed patterns in stem CH_4 and N_2O from the tropical species. These results are important as they show that tree stems in forests on free-draining soils can emit CH_4 even when there is seasonal CH_4 uptake in the surrounding soils, in agreement with studies of temperate upland forests (Wang *et al.*, 2016; Pitz and Megonigal, 2017; Maier *et al.*, 2017). The Wytham Woods study shows that tree stems emit CH_4 even after leaf fall in the winter, which could be important as this is also when soils were sources of CH_4. CH_4 fluxes measured at Wytham Woods were comparable to stem CH_4 fluxes measured at similar stem heights in temperate upland forests in the USA (Covey *et al.*, 2012, Pitz and Megonigal, 2017) and China (Wang *et al.*, 2016). There is no published data of *in situ* stem N_2O fluxes from trees in forests on free-draining soils and this novel data shows that accounting for emissions from tree stems could significantly increase estimates of the global forest source of N_2O. Seasonal responses

in stem CH_4 and N_2O were observed at multiple stem heights at Wytham Woods, confirming results from temperate wetlands and uplands; however these responses were not always the same between sampling heights.

At both sampling sites, exceptionally high fluxes of CH_4 and N_2O were measured from both tree stems and the soil surface. The regular occurrence of these outlier fluxes and their connection to temperature and moisture optima for both methanogenesis and denitrification implies that although these fluxes are statistical outliers they could represent "real" high flux events. Although inclusion of the outliers in statistical analyses of the effects of abiotic controls on CH_4 and N_2O fluxes obscured underlying relationships in the present work, further study of trace GHG fluxes from these forests could provide insights into predictable relationships with environmental variables that could improve GHG inventories from these ecosystems on a regional and global scale.

The studies presented in this thesis highlight a number of areas for future research into seasonal variation of CH_4 and N_2O fluxes in forests on free-draining soils.

- Our understanding of seasonal variation in stem CH_4 and N_2O fluxes could be improved by higher sampling frequencies. This would increase the opportunity to capture short-term wetting events which are known to increase rates of methanogenesis and denitrification (McClain *et al.*, 2003).
- Seasonal changes in tree stem CH_4 and N_2O fluxes may be better understood with a better monitoring of changes of soil trace greenhouse gas concentrations at a variety of depths (as per Maier *et al.*, 2017).
- Fluxes of CH_4 and N_2O were only sampled at a single height over the dry-to-wet transition in Panama and initially at three heights in Wytham. Increasing the number of heights sampled would enable a better understanding of seasonal changes in tree stem CH_4 and N_2O in both rainforests and temperate areas.
- Species common to all study plots were selected to aid reproducibility however given the diversity of species present, particularly in tropical rainforests, we still do not fully understand how species mixture can affect seasonal change in stem trace GHG fluxes. In Panama, across all the plots ~126 tree species were present of which only five were common to all the plots. At Wytham Woods, there are five dominant tree species so the low number of species sampled may be less of an issue.

6.2.2 Variation in tree stem CH_4 and N_2O fluxes with sampling height

Decreasing stem CH_4 fluxes with sampling height have been found in temperate and tropical wetland forests (Pangala *et al.* 2013; Pangala *et al.*, 2015) and upland forests (Wang *et al.*, 2016), but this thesis presents the first data on GHG fluxes from tree stems in forests on free-draining soils. The work described in Chapter Five, provides further evidence of decreases in tree-mediated

CH_4 and N_2O fluxes with sampling height. The results of the studies in Chapters Three and Four also demonstrated that tree stem CH_4 and N_2O uptake from temperate and tropical tree species on free-draining soils may be more common than currently thought. Tree stem uptake of CH_4 has been observed in only one other study to date, where 6 out of 68 CH_4 fluxes measured at 0.3-0.6-m stem height were weakly negative (Pitz and Megonigal, 2017).

Variation of tree stem CH_4 (Chapter 5) and N_2O (Appendix IV) fluxes with stem sampling height was investigated at the end of the wet season in Panama and during Winter at Wytham (Chapter 4). Stem fluxes of CH_4 decreased with sampling height at both sites Although N_2O was only sampled at 0.3-m and 1.3-m, there N_2O fluxes were lower at 1.3-m sampling height than at 0.3-m in Panama and there was a trend towards lower N_2O fluxes at 1.3-m in Wytham Woods. The decrease in trace GHG fluxes with height is consistent across all four species studied. The consistency of response between tropical and temperate forests suggests that the processes controlling variation in stem trace GHG fluxes with sampling height are the same globally and are not climate driven.

The work presented in this thesis demonstrates for the first time widespread tree stem uptake of trace GHGs. The average height at which tree stem CH_4 emissions pivoted from emission to uptake was consistent between the tropical (1.44±0.19-m) and temperate (1.31±0.15-m) sites. Some stem uptake of CH_4 was also recorded below 1-m sampling height in Wytham Woods (Fig. 4.5). Weak stem uptake of CH_4 was measured at 0.3-0.6-m sampling height from trees in a forested upland catchment in the USA (Pitz and Megonigal, 2017) and a study of Poplar trees in upland forests found fluxes of 0 μg CH_4 m^{-2} h^{-1} (Wang *et al.*, 2016) at stem sampling heights of 1.3-m. The present study makes an important contribution to research in this area because free-draining soils cover a larger area globally than wetlands, accounting for ~5% of the 632 Tg y^{-1} global annual CH_4 sink (Kirschke *et al.*, 2013). Studies of sources of CH_4 fluxes in a wetland forest in Indonesia estimated that 62-87% of ecosystem CH_4 was emitted from tree stems (Pangala *et al.*, 2013). Should tree stems in forests on free-draining soils account for a similar proportion of ecosystem CH_4 fluxes, then stem uptake of CH_4 above *c.* 1.3 - 2-m height (Fig. 5.1 & 5.2) could represent an important terrestrial CH_4 sink. Stem N_2O fluxes were highly variable at both sites, however unlike CH_4, mean N_2O fluxes from tree stems were positive at 1.3-m height.

The results presented in this thesis demonstrate an important gap in our knowledge of variation of tree-mediated N_2O fluxes and further work should aim to identify whether tree stems take up N_2O at greater stem heights. Traditionally stem greenhouse gas emissions are sampled at 1.3-m as that is where stem diameter is also measured. However as the results presented in thesis show, sampling at that height may not only lead to not capturing greater rates of stem CH_4 and N_2O efflux below that height but also stem uptake above that height. It is presently unknown how much of the CH_4 uptake by tree stems in forests on free-draining soils is from the free-atmosphere. It is possible that the negative CH_4 stem fluxes observed at 1.3-m and 2-m stem height are "recycling"

of CH_4 emitted lower down the stem in a repeating pattern of emission and uptake along the stem, rather than indicating a true sink for atmospheric CH_4. Until the mechanism of tree stem uptake has been established, estimates of tree stem contributions to ecosystem exchange of trace GHGs remain uncertain. To establish whether the majority of mature tree stems are indeed a sink of CH_4 and N_2O, long-term sampling of stem trace GHG fluxes at multiple heights (at regular intervals up to five metres or more) is needed in future.

6.2.3 Inter-species variation in stem CH_4 and N_2O fluxes

Observations of interspecific differences in tree stem CH_4 and N_2O fluxes have been made in upland and wetland forests (Covey *et al.*, 2012; Pangala *et al.*, 2013; Wang *et al.*, 2016; Pitz and Megonigal, 2017) or in mesocosm studies, which may not be representative of natural systems (Machacova *et al.*, 2013). In the work presented in Chapter Three, there were no clear species effects on tree stem CH_4 and N_2O fluxes during the 2014 dry-to-wet transition in the tropical forest in Panama. However, interactions between species and litter manipulation appeared to alter stem trace GHG fluxes. Stem CH_4 fluxes from *Heisteria* were greater than those from *Simarouba* in litter addition plots (Fig. 3.4) but stem N_2O fluxes from *Heisteria* were lower than those from *Simarouba* (Fig. 3.10). The finding that there were no overall effect of species was unexpected as it was hypothesised that the fast growing canopy species *Simarouba* would have greater stem GHG fluxes than the slow-growing subcanopy species *Heisteria*. Litter manipulation experiments are used to stimulate increased NPP and consequently leaf fall. The observed interaction between species and the litter addition treatment implies that the amount of litter on the forest floor can enhance inter-species differences in stem fluxes. Therefore, species mixture could play an important role in determining the sink potential of CH_4 in managed tropical forests, particularly if stem trace GHG fluxes are greater from species commonly planted in mono-culture plantations for forestry and agricultural purposes.

At Wytham Woods, stem CH_4 but not N_2O fluxes differed between species. Stem CH_4 fluxes at 0.3-m were marginally higher from ash than from Sycamore trees but stem CH_4 fluxes were lower for ash than Sycamore at greater sampling heights. Similar interspecific differences were reported for upland forests in Maryland, USA however as these stem CH_4 fluxes were only sampled at breast height changes with sampling height are unknown (Warner *et al.*, 2017). Differences in wood specific density between species can be a significant control of GHG fluxes in a tropical wetland forest, with higher stem CH_4 efflux in species with lower wood density (Pangala *et al.*, 2013). However, in the present study, stem CH_4 fluxes were lower in the species with lower wood density in Panama and the difference in wood specific density between Ash and Sycamore trees at Wytham was negligible (0.04 g cm^{-3}; Wiemann *et al.*, 2007). Differences in gas exchange structures, such as lenticels, between species may also affect stem trace GHG fluxes. Stem CH_4

fluxes increased with stem lenticel density (Pangala *et al.*, 2013) and may be why tree stems in a temperate upland forest increased with sampling height (Maier *et al.*, 2017).

For some time heartwood rot has been known to occur in a significant proportion of trees (Wagener and Davidson, 1954). Microbial production of super-ambient CH_4 within tree stems has been mooted as a potential source of stem CH_4 in upland trees, especially at stem heights of ~1.3-m (Covey *et al.*, 2012; Wang *et al.*, 2016). None of the trees in this study were surveyed for heartwood rot and so the contribution of inter-species differences in decay resistance (Scheffer and Cowling, 1966) to the significantly different stem CH_4 fluxes between study species cannot be quantified.

Mesocosm and field studies have shown that tree species affect production of CH_4 and N_2O in the soil by influencing soil physical structure (Fender *et al.*, 2013a), litter decay rates (Aubert *et al.*, 2010) and soil chemistry (Hanson and Hanson, 1996; Augusto *et al.*, 2002; Hagen-Thorn *et al.*, 2004). Differences in tree physiology can also affect stem CH_4 efflux, especially in wetland adapted species (Pangala *et al.*, 2013) but questions remain about how physiology of tree species that grow on free-draining soils could influence stem trace GHG fluxes. There is a need to develop models of ecosystem CH_4 and N_2O fluxes that account for species mixtures without assuming commonality between species. The results presented in this thesis reinforce the need for further research into species-specific differences in tree stem CH_4 and N_2O fluxes, especially in light of possible future ecological changes such as Ash die-back in the UK.

6.2.4 Litter manipulation effects

Chapter Three presented the first study to examine the effects of litter manipulation on soil and tree stem CH_4 and N_2O fluxes. Soil and tree stem CH_4 and N_2O fluxes were hypothesised to be lower in litter removal plots and higher in litter addition plots. Litter addition increased fluxes of soil N_2O (Fig. 3.9), as well as tree stem CH_4 (Fig. 3.4) and N_2O (Fig. 3.10) fluxes. Unexpectedly however, the effects of litter manipulation were only apparent when tree species identity was also taken into account. Increases in forest NPP with elevated atmospheric CO_2 also increase litter production (Liu *et al.*, 2005). Litter manipulation simulates the effect of future atmospheric conditions and the related increase in NPP, which in turn increases the availability of acetate and nitrate for methanogenesis and denitrification (Sayer, 2006). Therefore it was expected that litter manipulation effects should have been universal.. The lack of significant effect of litter manipulation on soil CH_4 fluxes was surprising but most likely explained by SWC being too low for soil surface CH_4 fluxes to be affected by changes in acetate availability (Teh *et al.*, 2008). The interaction between tree species and litter treatment shows that multiple factors such as water utilisation, NPP and tree physiology could influence the overall impact of global change on CH_4 and N_2O fluxes from forest ecosystems.

Fertilizer addition experiments have been performed in tropical rainforests previously that lead to a doubling of soil N_2O fluxes and an increase in soil CH_4 concentrations however such experiments typically use unnaturally high nitrogen inputs (Koehler *et al.*, 2009). Pivot point heights were greater from both study species in litter addition plots, most likely because there was more source material for methanogenesis, increasing soil CH_4 concentrations. As rates of methanogenesis increase this could potentially increase the surface area for stem CH_4 emission in the future as it will take longer for internal CH_4 concentrations to reach ambient levels (Appendix III). However, a comparison of litter manipulation and fertilizer addition effects on the Gigante Peninsula found that plant uptake of nutrients was greater in litter addition plots than fertilized plots (Sayer *et al.*, 2012). The implication of this for trace GHG fluxes is that greater amounts of CH_4 and N_2O could be transported from soils into tree stems. This could raise pivot point heights, increasing the tree stem surface area for CH_4 and N_2O emission. Over the longer term increased litter inputs could therefore reduce tree stem CH_4 uptake. Without synchronously collected data on important influencing factors such as decomposition rates, fine root biomass and root exudates, it is hard to define how exactly soil and tree stem trace GHG fluxes are affected by litter manipulation. As the interaction between trees and soils is a key component of tree-mediated CH_4 and N_2O emissions and uptake, further research into the effect of litter manipulation on tree stem fluxes is required, especially in temperate forests on free-draining soils.

6.3 Implications

The majority of mature hardwood trees found in tropical and temperate forests have stem heights in excess of 10-m. The results presented in this thesis demonstrate that the majority of stem CH_4 emissions are from the lower 1-1.5-m of stems. If the rates of tree stem CH_4 uptake measured at 1.3-m and 2-m in this body of work are representative of CH_4 uptake in other tropical and temperate forests, the global terrestrial CH_4 sink may be greater than presently assumed.

Tree stem CH_4 emissions in other forests on free-draining soil are estimated to offset 3.5% to 63% of soil CH_4 uptake (Wang *et al.*, 2016; Pitz and Megonigal, 2017; Warner *et al.*, 2017). However none of the above studies measured tree stem CH_4 uptake and in one case tree stem CH_4 fluxes were only measured at 0.3-0.6-m stem height (Pitz and Megonigal, 2017). The data presented in Chapters 4 and 5 demonstrate tree stem uptake of CH_4 from tropical and temperate forests on free-draining soils and as such, tree stem CH_4 uptake at stem heights over 1.3-m may not offset soil CH_4 uptake but rather enhance rates of CH_4 uptake at an ecosystem scale.

In Chapter 5, ecosystem CH_4 fluxes at both Panama and Wytham were estimated by extrapolating fluxes sampled at 0.3-m, 0.75-m, 1.3-m and 2-m stem height to the lower 2.5-m and 15-m of tree stems. Based on the mean estimated values from the study sites, and using Pan *et al.'s* (2013) estimated global areas for deciduous moist tropical forests (795 M ha) and continental temperate forests (473 M ha), global tree stem CH_4 fluxes up to 15-m height amount to -1.75±1.80

Tg CH_4 yr^{-1} and 1.32±1.78 Tg CH_4 yr^{-1}, respectively. Accordingly, global estimates for soils in these biomes are -0.11±1.48 Tg CH_4 yr^{-1} and 0.27±0.95 Tg CH_4 yr^{-1}, respectively. This would mean that tropical moist forests act as a global CH_4 sink, with an uptake of -1.86 ±3.28 Tg CH_4 yr^{-1}, whereas continental temperate forests are global CH_4 sources, with emissions of 1.59 ±2.73 Tg CH_4 yr^{-1}. However, if these values are applied to other areas of global tropical forest on free-draining soils (e.g. rain forest, dry forest and mountain forest systems) the tropical forest tree sink is -7.38±7.62 Tg CH_4 yr^{-1} globally (based on a tropical forest area of 3145 M ha). Tropical forest tree uptake of CH_4 exceeds the emissions from methane hydrates and permafrost which is estimated to be ~7 Tg yr^{-1} which would be sufficient to almost negate the imbalance between top-down estimated sources and sinks (Kirschke *et al.*, 2013).

Ecosystem-level N_2O fluxes from tree stems were not estimated because although mean stem N_2O fluxes decreased significantly with sampling height at both sites, it is not known whether there is any N_2O uptake at greater stem heights. Additionally at Wytham there were differences in estimated error. The model error was much lower for the period between October 2015 and January 2016 because the model was based on measured CH_4 fluxes for 2-2.5-m stem height, rather than estimates. It is therefore possible that the model overestimated CH_4 fluxes up to 15-m stem height for February to September 2015 due to derivation of fluxes at 2-m. These estimates show need to better understand the interactions between trees and soil chemical cycles in regulation of global CH_4 and N_2O.

6.4 Recommendations for future work

The results presented in this thesis show that tree stem fluxes in forests on free-draining soils may contribute significantly to the global biosphere-atmosphere exchange of CH_4 and N_2O. Several key questions remain to be answered, in particular regarding the transportation mechanisms and biogeochemical processes that control fluxes in these ecosystems:

- In both tropical and temperate forest on free-draining soils, tree stems show CH_4 uptake and decreased N_2O emissions from ~1.3-m stem height. Upscaling from individual tree stem fluxes to plot, regional and ecosystem fluxes could be improved by sampling trace GHG fluxes at regular intervals to at least 5-m stem height. This would contribute to the development of a standard model and a better understanding of variation in stem trace GHG flux emissions.
- Heartwood rot can generate significant quantities of CH_4 in temperate upland trees (Covey *et al.*, 2012; Wang *et al.*, 2016) and may underlie some of the extreme outliers observed in the studies presented in this thesis (APPENDICES I, II, III and IV). However the prevalence of heartwood rot and its relative contribution to stem CH_4 fluxes is not fully understood. Cores of stem tissue taken at multiple stem heights following stem trace GHG

flux sampling could be incubated under varying atmospheric conditions to quantify rates of methanotrophy and methanogenesis.

- In addition to investigating spatial variation of the contributions of methanotrophy and methanogenesis to tree stem CH_4 fluxes, it would be prudent to assess variations in microbial communities within stems and between tree species. If certain species can be identified as having larger methanogenic populations, afforestation methods may seek to plant species that will increase absorption of atmospheric CH_4.
- Litter quantity has been shown to be a significant control of soil and tree stem CH_4 and N_2O fluxes in tropical forests on free-draining soils. A similar study in temperate forest on free-draining soils would establish whether responses are consistent between climate zones. How litter quality and mineralization rates affect stem CH_4 and N_2O fluxes could be assessed using different litter mixtures.
- CH_4 and N_2O fluxes varied significantly between species but stem fluxes were only sampled from two species per site. Stem trace GHG fluxes should be sampled from a wider range of species in both tropical and temperate forests. This would aid estimation of CH_4 and N_2O fluxes from these forests as well as help to predict how changes in species composition and climate could affect trace GHG fluxes.
- Results from this study demonstrate a need to better understand how the interaction of tree root systems and soil microbial communities affects both soil and tree stem CH_4 and N_2O fluxes. The role of root structure, exudates and the rhizosphere (fungal populations in particular) and their effects on soil methanogenesis, denitrification and transport of trace GHG from soils into tree stems merits further attention.
- Stem CH_4 fluxes at 1.3-m increased significantly with solar radiation in Wytham Woods. Gross Primary Productivity (GPP) was strongly related to soil CH_4 efflux in a temperate evergreen forest in the USA (Shoemaker *et al.*, 2014). Further studies are required to establish whether there is a causal link between stem trace GHG fluxes and solar radiation (and by extension GPP and NPP). This would likely require simultaneous measurements of solar radiation, stem trace GHG fluxes and concentrations of CH_4 and N_2O in sap flow and in the soil at multiple depths.

6.5 Conclusion

This thesis presents the first studies of trace GHG fluxes from tree stems in tropical and temperate forests on free-draining soils, as well as the first *in situ* study of N_2O fluxes from tree stems in a tropical ecosystem. The collective results suggest that tree stems in tropical and temperate forests on free-draining soils may represent a hitherto unaccounted for sink of CH_4.

To date, estimates of the global CH_4 terrestrial sink and N_2O source provided by aerated forest soils have not included the potential contributions of fluxes from overlying vegetation. More research is needed to better understand the processes at play, but the results presented in this thesis lay a foundation for further work and demonstrate the need to account for the tree-mediated exchange of CH_4 and N_2O. Given that forests on free-draining soils cover a larger area than wetland forests, the contribution of stem CH_4 and N_2O fluxes to ecosystems and global trace GHG budgets could be globally significant and merits further study.

Appendix I: Chapter 3 Results including outliers

Seasonal variation in CH_4 fluxes

Soil CH_4 fluxes varied significantly between the dry and wet season ($p < 0.001$, $r^2 = 0.463$, $\chi^2 = 77$; Fig. 1.a), whereby soils acted as a methane sink during the dry season and switched to being a source within 2-3 weeks of the start of the wet season. There was no clear seasonal pattern for CH_4 fluxes from tree stems; although stem CH_4 fluxes appeared to be larger during the wet season these are distorted by a few outlying flux values (Fig. 1).

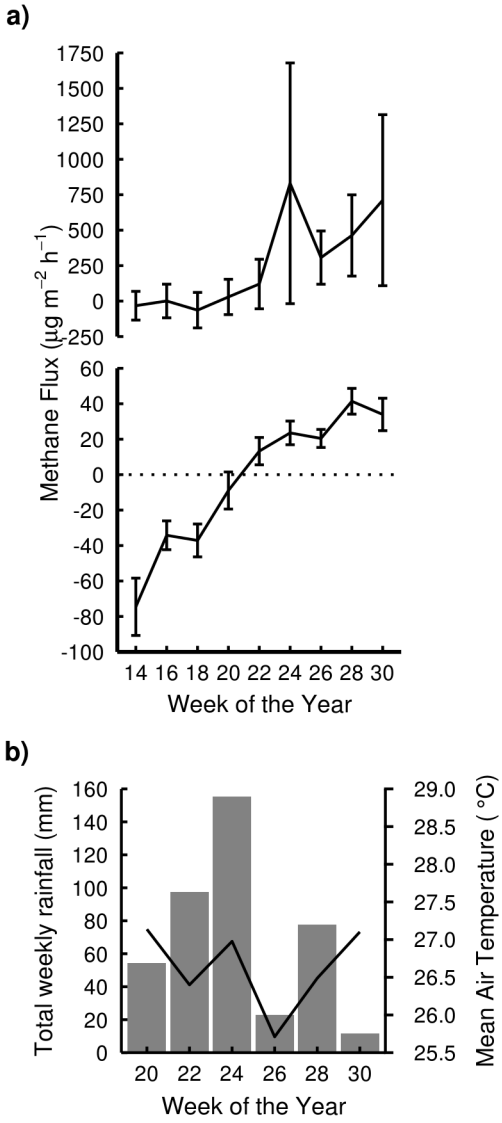

Figure 1. a) Seasonal patterns of methane (CH_4) fluxes from tree stems (top panel) and soil (bottom panel) in a lowland tropical forest on free-draining soil in Panama, Central America, showing weekly mean stem fluxes measured at 0.3-m height, and weekly mean soil fluxes measured over chambers during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30); error bars show the standard error means for $n = 4$; b) Total rainfall in the week of sampling measured at a rainfall gauge on BCI (bars) and air temperature measured in the plots during gas sampling (line). Tree stem fluxes are the mean of all fluxes pooled across species and litter treatment.

Soil chamber fluxes of CH_4

Soil CH_4 fluxes measured over chambers under individuals of *Heisteria* remained predominantly negative until week 24 of sampling, indicating dry season uptake of CH_4 before transitioning to positive fluxes (i.e. emission) four weeks after the first heavy rainfall of the year (Fig. 2). Soil CH_4 fluxes underneath *Simarouba* became positive two weeks after the first heavy rains in week 22. The median flux beneath *Heisteria* was 8.33 µg m^{-2} hr^{-1}, which is slightly higher than the median CH_4 flux of 6.25 µg m^{-2} hr^{-1} from chambers under *Simarouba*.

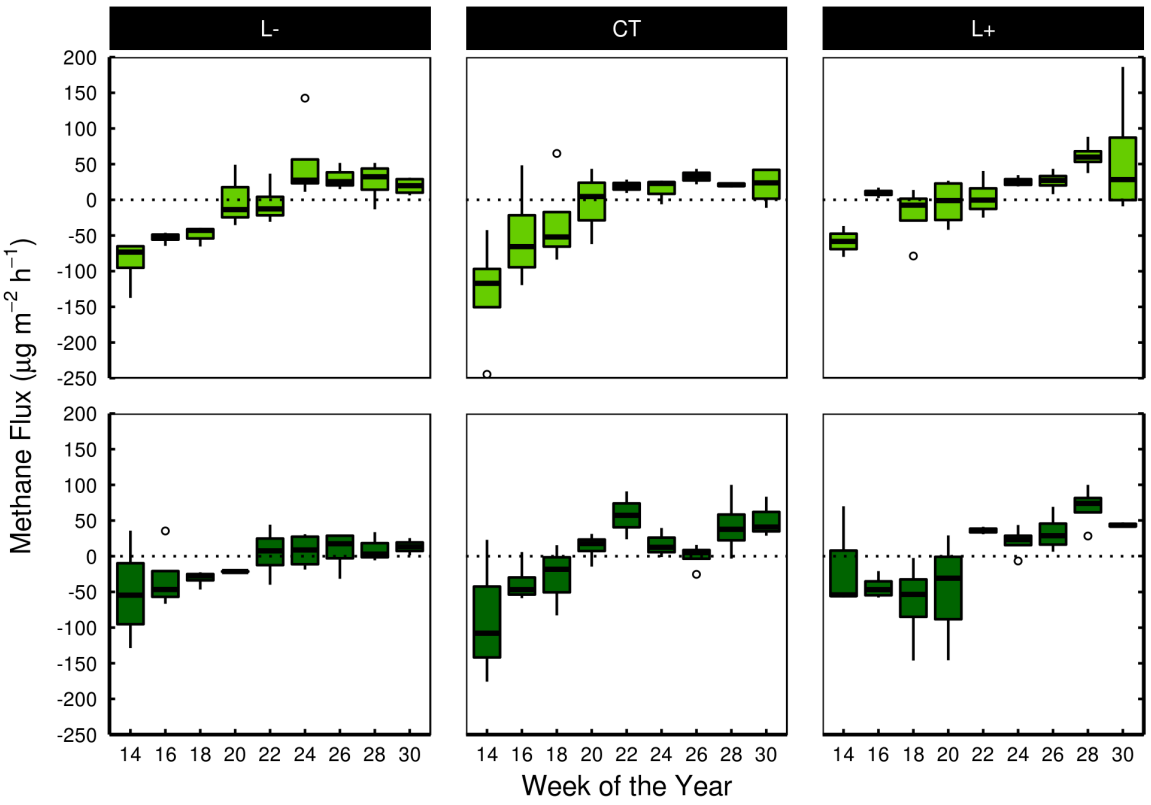

Figure 2. Weekly ranges of soil methane (CH_4) fluxes measured over chambers under individuals of two common tree species: Heisteria concinna (light green, top panels) and Simarouba amara (dark green, bottom panels) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30), showing the ranges (boxes and whiskers) and median lines for *n* = 4 individuals per species and treatment. Ranges are based on four replicates per species.

During the study, soil CH_4 fluxes under individuals of *Heisteria* had a greater range (-245 - 190 µg m^{-2} hr^{-1}) than under individuals of *Simarouba* (-176 - 100 µg m^{-2} hr^{-1}). Consequently, the mean soil CH_4 flux beneath *Heisteria* individuals was marginally more negative than that beneath *Simarouba* (-3.32 ±5.98 µg m^{-2} hr^{-1} and -2.29 ±5.46 µg m^{-2} hr^{-1}, respectively). There were no effects of species, treatment, or their interaction on soil CH_4 fluxes.

Tree stem fluxes of CH_4

Surprisingly, tree stem CH_4 fluxes were mostly positive throughout the study period, indicating that tropical trees emit CH_4 even when they are growing on free-draining soils. There were no significant differences in stem fluxes of CH_4 between tree species and no overall effects of litter manipulation on stem CH_4 fluxes. Nor was there a species × treatment interaction. Overall, the median CH_4 flux was higher from *Heisteria* stems than *Simarouba* stems, with 119 µg m^{-2} hr^{-1} and 86.5 µg m^{-2} hr^{-1} for *Heisteria* and *Simarouba*, respectively. Tree stem CH_4 fluxes in individuals of *Heisteria* were mostly positive, with a mean flux of 466 ±249 µg m^{-2} hr^{-1} over the dry-wet season transition however the mean flux for *Simarouba* was much lower at 66.4 ±27.6 µg m^{-2} hr^{-1}. The median stem flux remained relatively constant throughout the study (Fig. 3).

Stem CH_4 fluxes in *Simarouba* displayed greater inter-week variability (Fig. 3.3) than those from *Heisteria* stems but there were many more outliers in *Heisteria* (Appendix 1). CH_4 stem fluxes from *Heisteria* ranged from -2347 to 19350 µg m^{-2} hr^{-1}, *Simarouba* had a smaller range throughout the study, ranging from a low of -1110 µg m^{-2} hr^{-1} to a maximum of 1147 µg m^{-2} hr^{-1}.

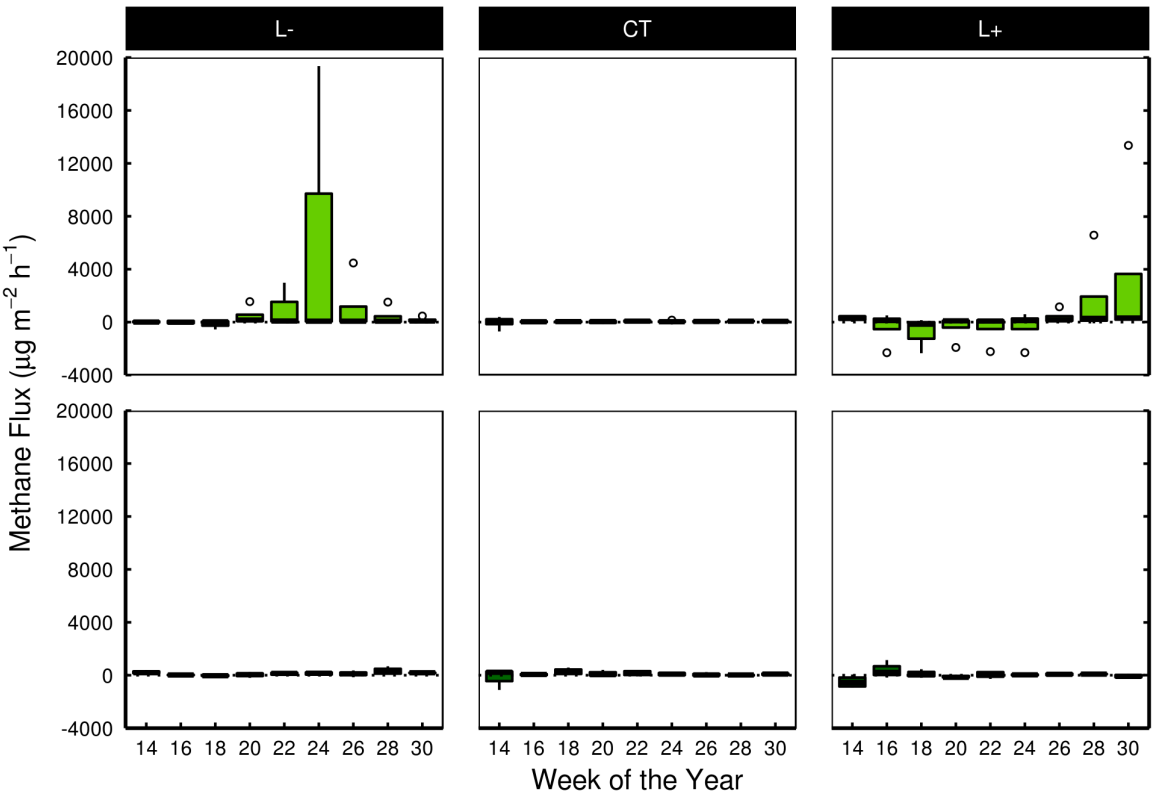

Figure 3. Weekly methane (CH_4) fluxes from tree stems in a lowland tropical forest in experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, top panels) and *Simarouba amara* (dark green, bottom panels), during the transition from the dry season (weeks 14-19) to the wet season (weeks 20-30), showing the ranges (boxes and whiskers) and median lines for *n* = 4 individuals per species and treatment. Ranges are based on four replicates per species.

Controls of soil and stem CH_4 fluxes

Soil CH_4 fluxes tended to increase with soil water content (Fig. 6) but there was no significant relationship between soil CH_4 fluxes and soil temperature, air temperature (Figs. 4 & 5) or rainfall. Tree stem fluxes had no significant relationship with soil temperature, air temperature, rainfall or solar radiation.

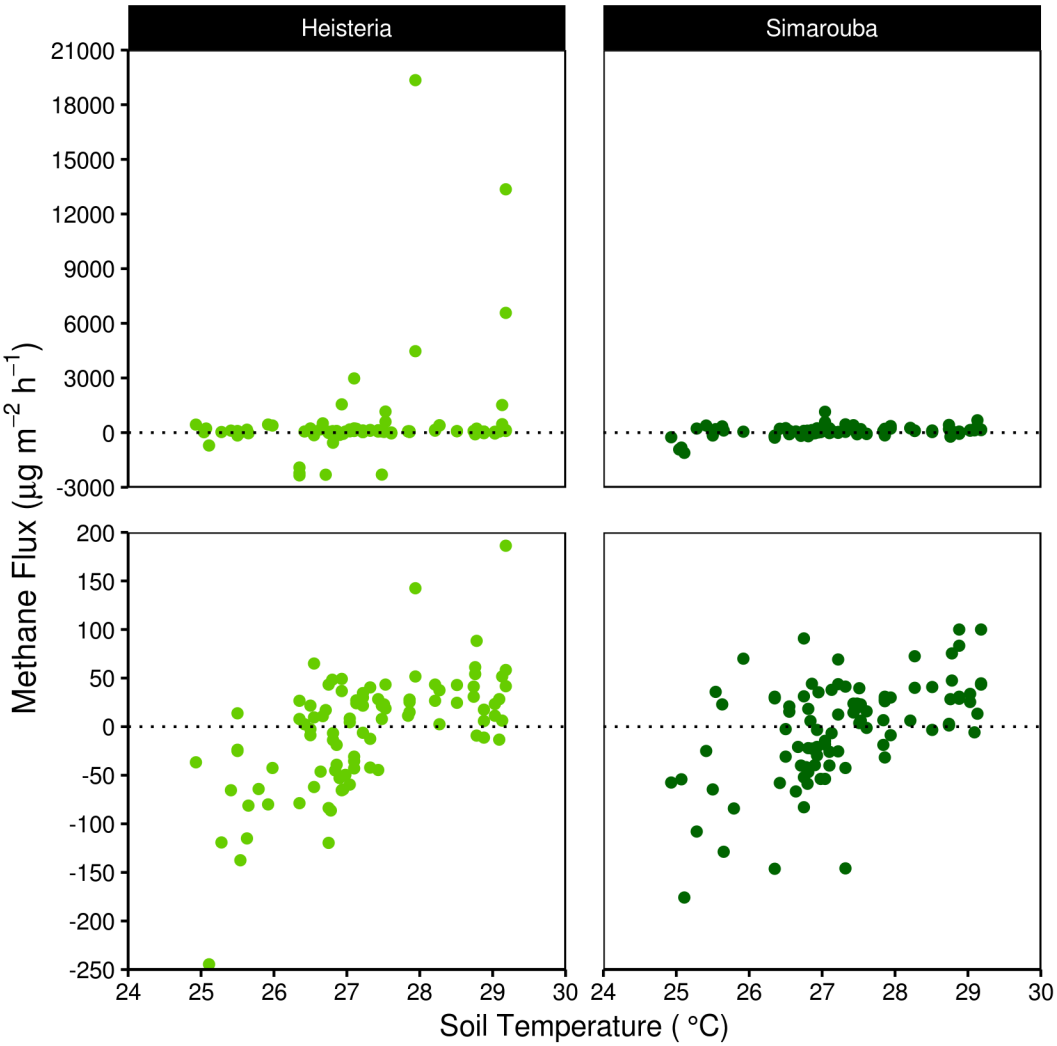

Figure 4. Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, left) and *Simarouba amara* (dark green, right) and the mean flux of two chambers beneath each tree, from March to July 2014.

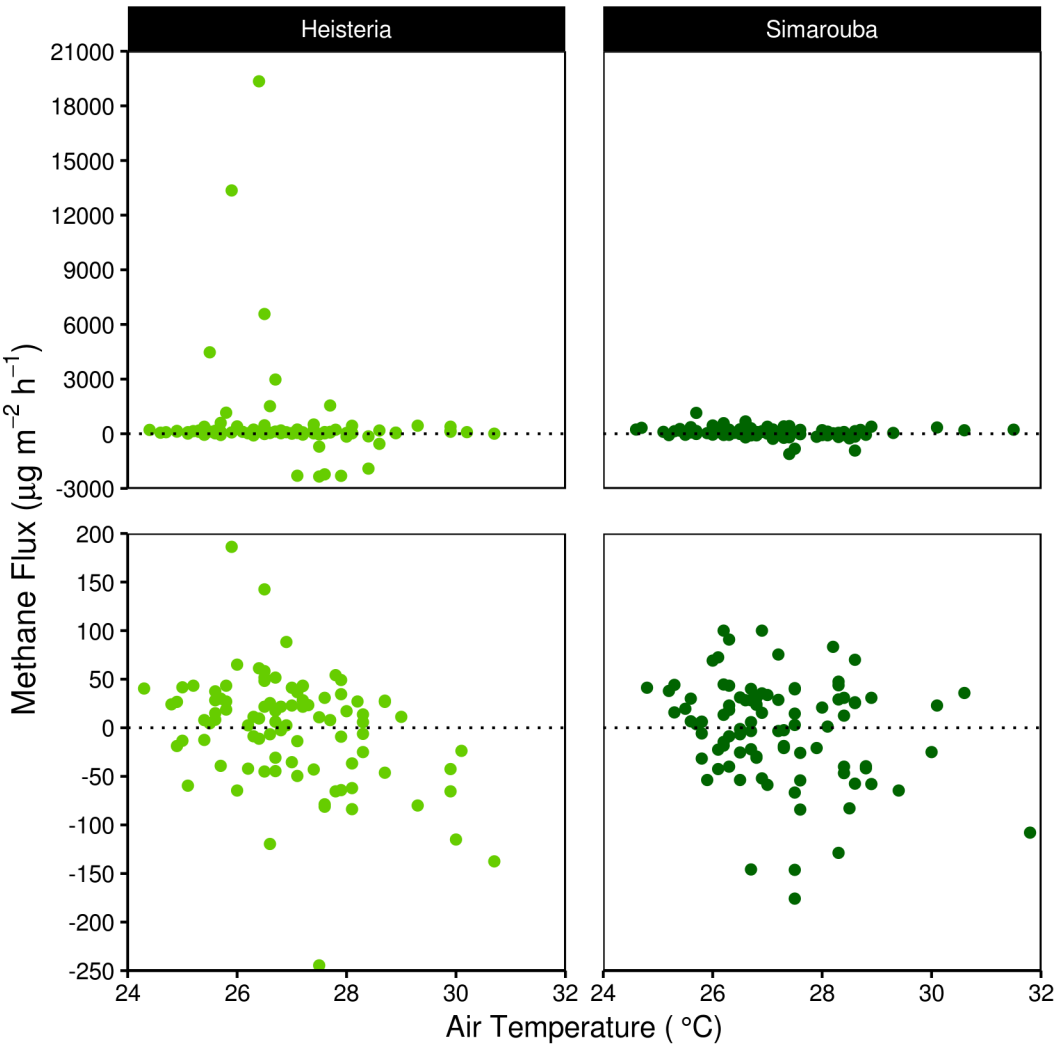

Figure 5. Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and air temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, left) and *Simarouba amara* (dark green, right) and the mean flux of two chambers beneath each tree, from March to July 2014.

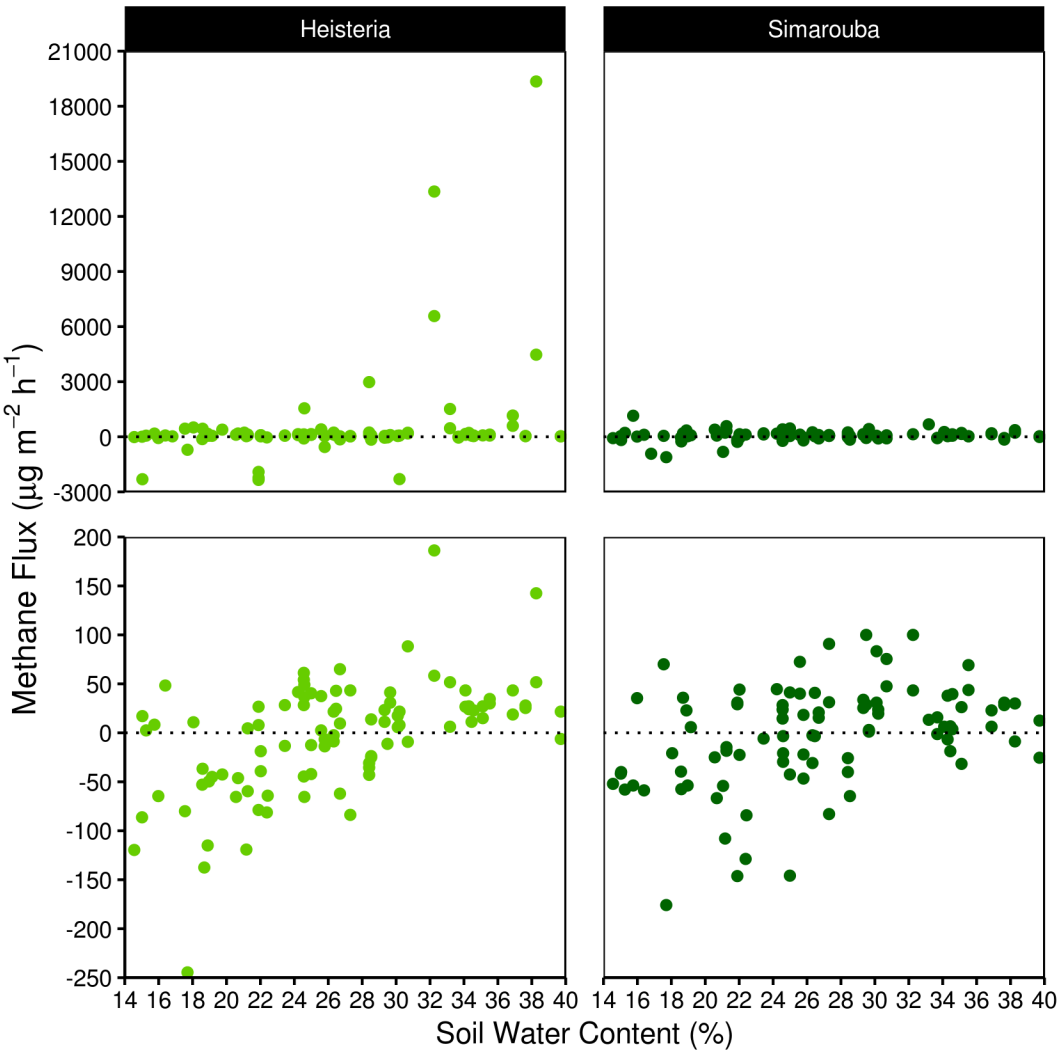

Figure 6. Scatter plots of the relationship between methane (CH_4) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil water content in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, left) and *Simarouba amara* (dark green, right) and the mean flux of two chambers beneath each tree, from March to July 2014.

Seasonal variation of N_2O fluxes

There was no clear season pattern in tree stem or soil N_2O fluxes during the study period but N_2O concentrations in air samples collected from the soil collars during the dry season were mostly below the limits of detection.

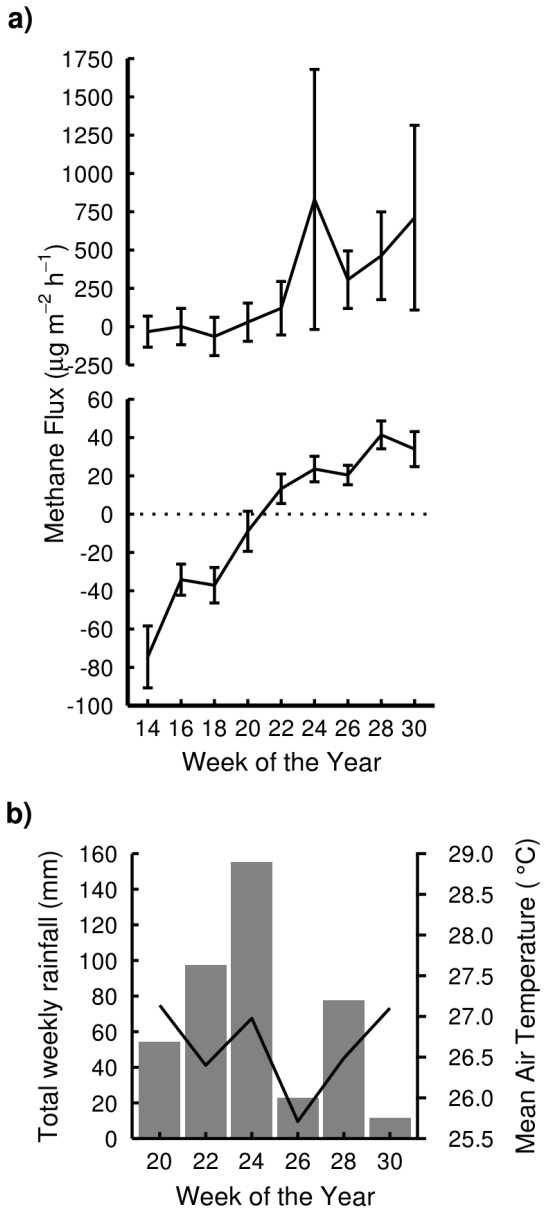

Figure 7. a) Seasonal patterns of nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil (bottom panel) in a lowland tropical forest on free-draining soil in Panama, Central America, showing weekly mean stem fluxes measured at 0.3-m height, and weekly mean soil fluxes measured over chambers during the wet season (weeks 20-30); concentrations of N_2O in dry season samples were below the limits of detection; error bars show the standard error means for n = 4; **b)** Total rainfall in the week of sampling measured at a rainfall gauge on BCI (bars) and air temperature measured in the plots during gas sampling (line). Tree stem N_2O fluxes shown are the mean of pooled N_2O fluxes across species and litter treatment.

Soil chamber fluxes of N_2O

Median soil N_2O fluxes from chambers beneath *Heisteria* (119 µg m^{-2} h^{-1}) and *Simarouba* (86.5 µg m^{-2} h^{-1}) were lower than the median stem fluxes. Fluxes beneath *Heisteria* individuals ranged from -190 to 539 µg m^{-2} h^{-1} with fluxes under *Simarouba* trees ranging from -585 to 450 µg m^{-2} h^{-1}. During the wet season, mean soil N_2O fluxes were 138 ±21.3 µg m^{-2} h^{-1} beneath *Heisteria* and 101 ±20.3 µg m^{-2} h^{-1} beneath *Simarouba*. There were no significant species, treatment or interaction effects on soil N_2O fluxes (Fig. 8).

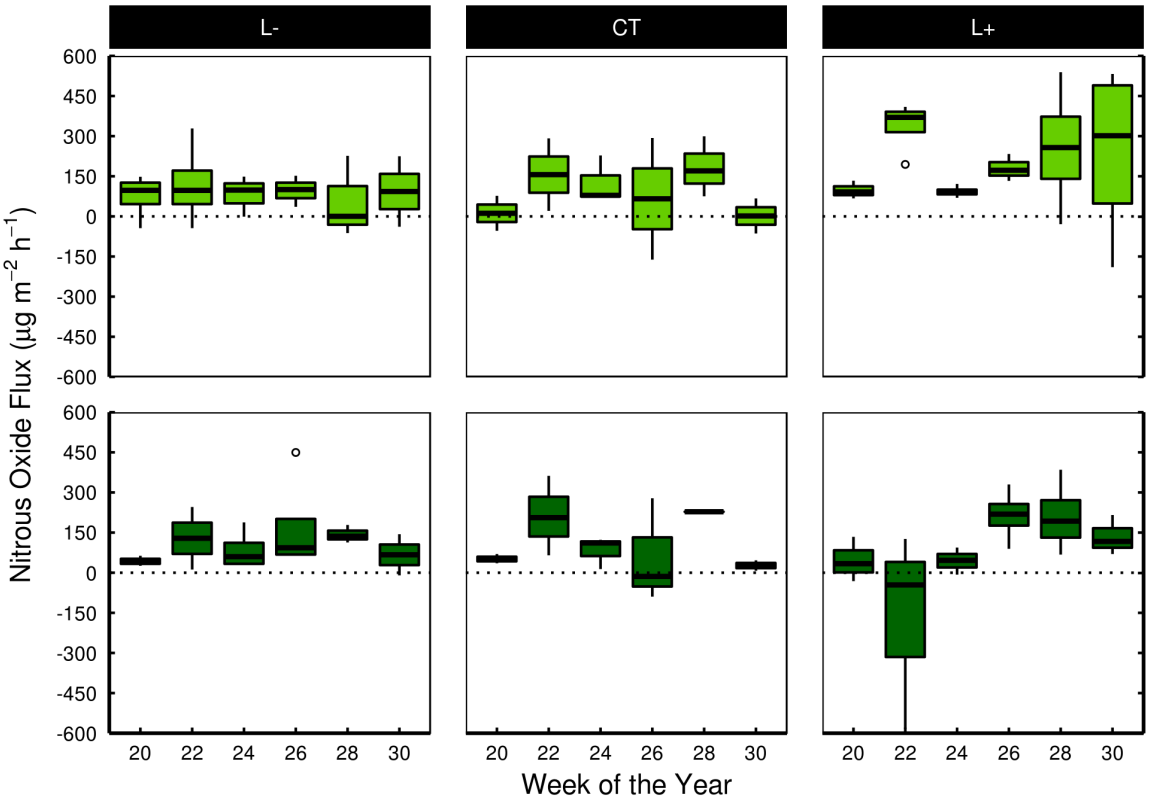

Figure 8. Weekly ranges of soil N_2O fluxes measured over chambers under individuals of two common tree species *Heisteria concinna* (pale green, top panels) and *Simarouba amara* (dark green, bottom panels) in a lowland tropical forest with experimental litter manipulation (litter removal (L-), control (CT) and litter addition (L+)) on free-draining soil in Panama, Central America, during the wet season. Dry season data is not present as N_2O concentrations in dry season air samples were below the limits of detection. Ranges are based on four replicates per species.

Tree stem fluxes of N_2O

Although there were no significant differences in stem N_2O fluxes between species, median N_2O fluxes from *Heisteria* were much lower than those from *Simarouba* (222 µg m^{-2} h^{-1} and 894 µg m^{-2} h^{-1}, respectively) over the course of the study. N_2O fluxes from *Heisteria* stems tended to be lower (range: -2857 to 15657 µg m^{-2} h^{-1}) compared to *Simarouba* (range: -10425 to 8361 µg m^{-2} h^{1}; Fig. 9). Overall, a greater proportion of stem fluxes in *Simarouba* were positive and the mean stem flux from *Heisteria* was 519 ±345 µg m^{-2} h^{-1} compared to 854 ±424 µg m^{-2} h^{-1} for *Simarouba*. There was a marginal effect of species × treatment interaction ($p < 0.1$, $\chi^2 = 7.27$; Fig. 9).

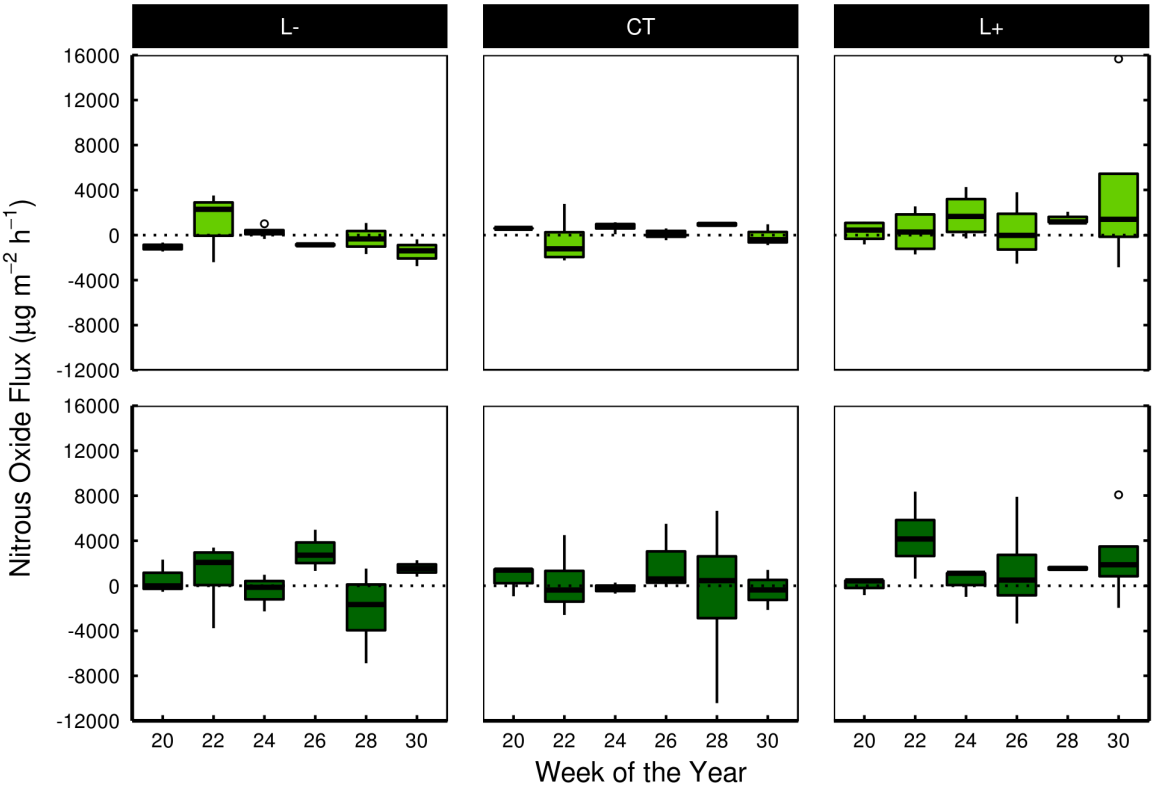

Figure 9. Box and whisker plot of N_2O fluxes from tree stems in a lowland tropical forest with experimental litter manipulation (litter removal (L-), control (CT) and litter addition (L+)) on free-draining soil in Panama, Central America, showing stem fluxes in two common tree species *Heisteria concinna* (pale green, top panels) and *Simarouba amara* (dark green, bottom panels), measured at 0.30-m height during the wet season. Dry season data is not shown as N_2O concentrations in dry season air samples were below the limits of detection. Ranges are based on four replicates per species.

Controls of tree stem and soil N_2O

Similar to CH_4 fluxes, there was no clear relationship between tree stem or soil N_2O fluxes and soil temperature (Fig. 10) or air temperature (Fig. 11). In addition, tree stem fluxes were not significantly related to weekly mean solar radiation or soil water content (Fig. 12).

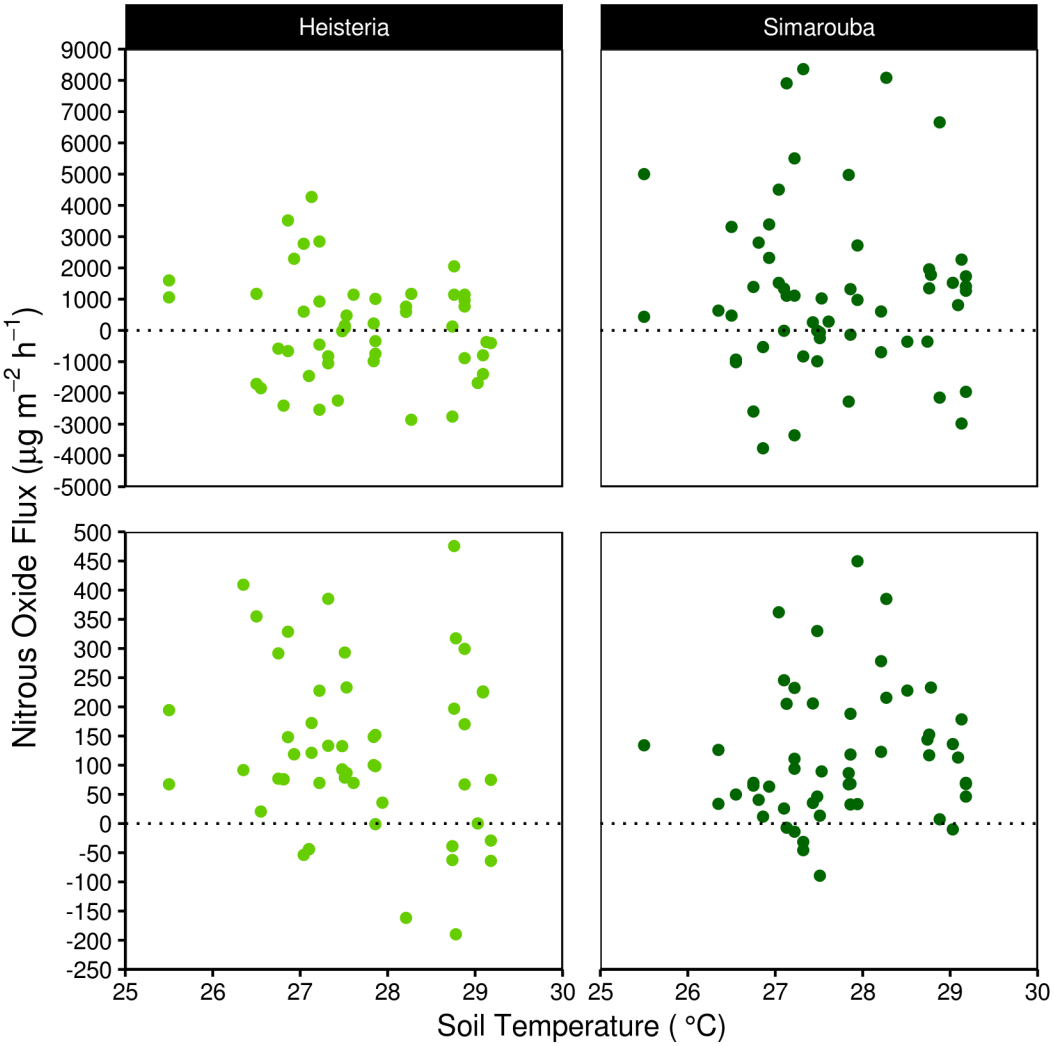

Figure 10. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, left) and *Simarouba amara* (dark green, right) and the mean flux of two chambers beneath each tree, from May to July 2014.

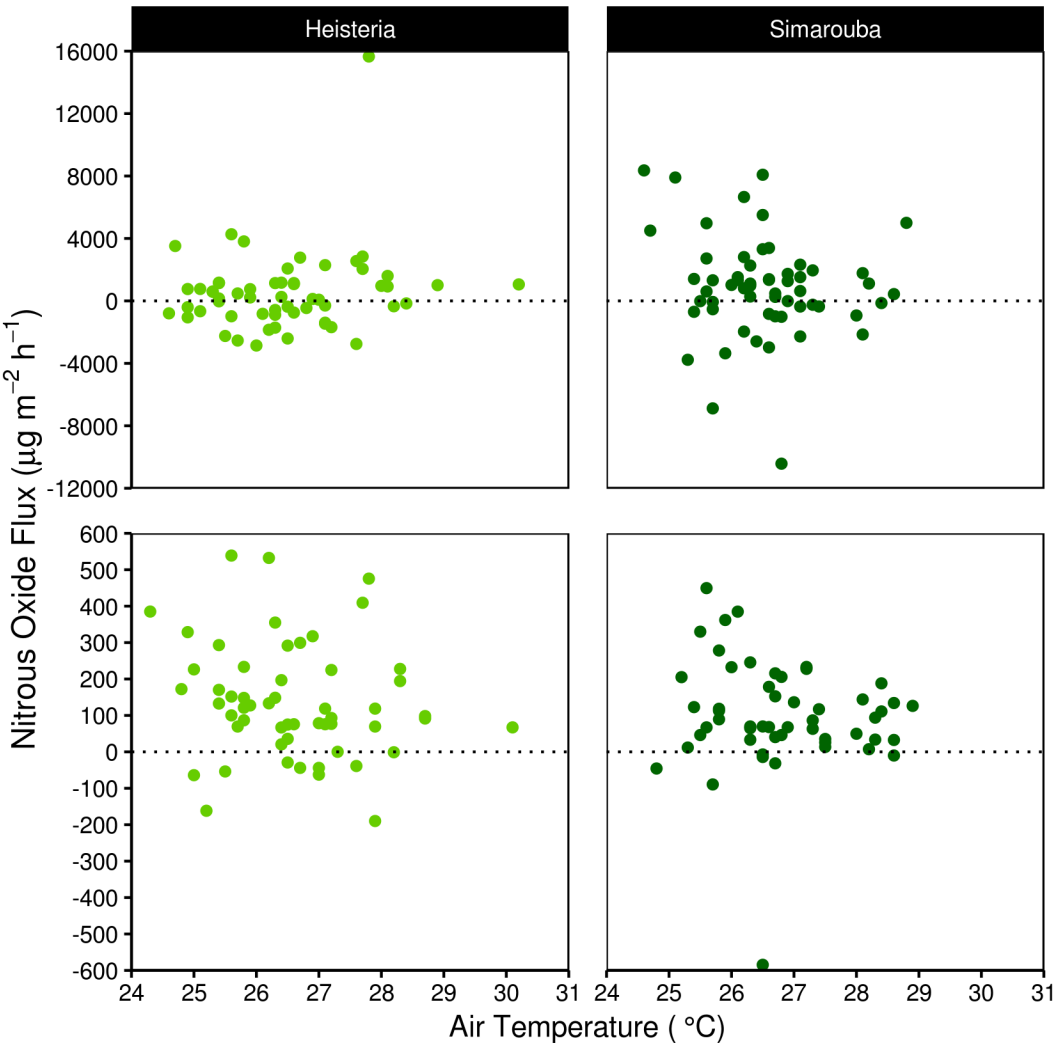

Figure 11. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and air temperature in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: *Heisteria concinna* (pale green, left) and *Simarouba amara* (dark green, right) and the mean flux of two chambers beneath each tree, from May to July 2014.

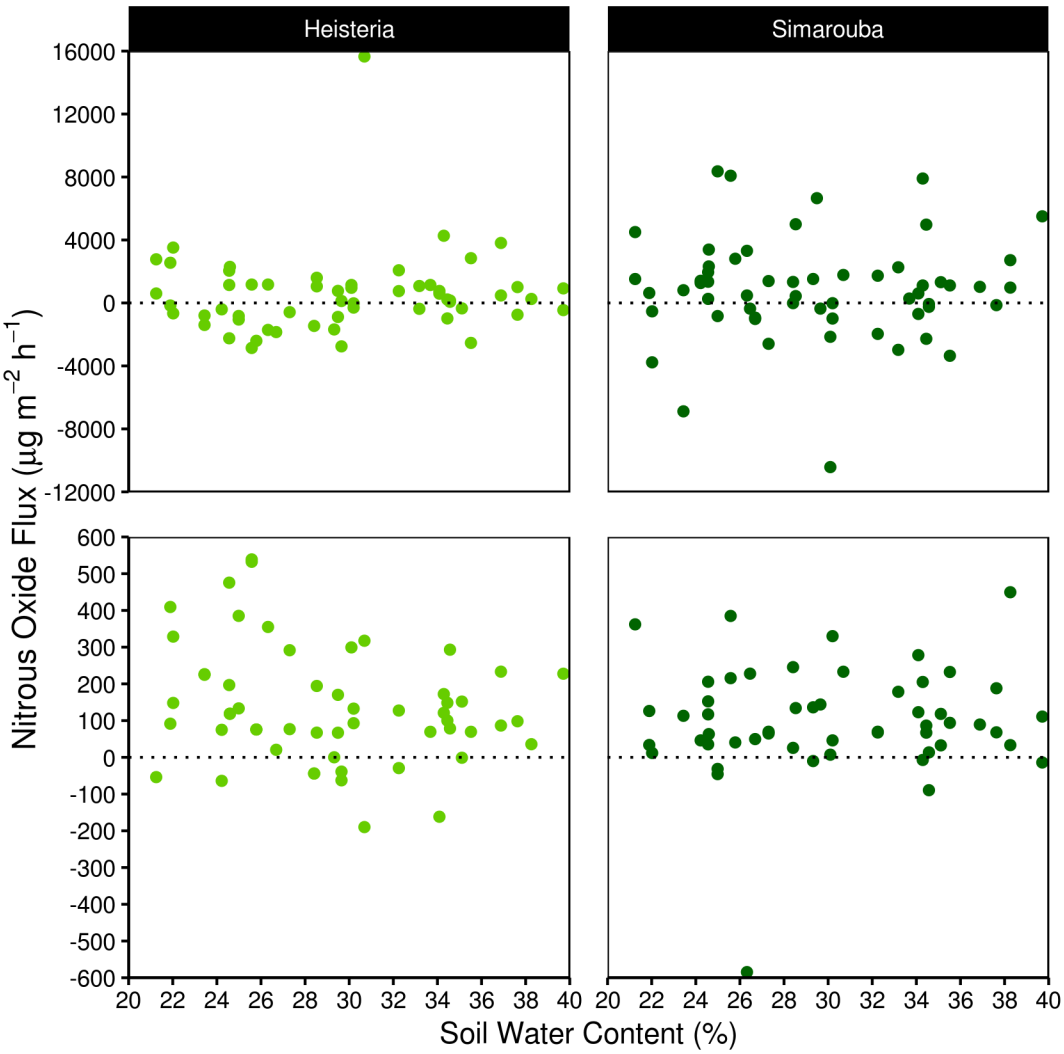

Figure 12. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems (top panel) and soil chambers (bottom panel) and soil water content in a lowland tropical forest with experimental litter removal (L-), control (CT) and litter addition (L+) treatments on free-draining soil in Panama, Central America, showing stem fluxes measured at 0.3-m height in two common tree species: Heisteria concinna (pale green, left) and Simarouba amara (dark green, right) and the mean flux of two chambers beneath each tree, from May to July 2014.

Appendix II: Tree stem CH_4 fluxes in Wytham Woods Oct. 2015 – Jan. 2016

Seasonal variation in CH_4 fluxes

Stem fluxes were only measured at 2.00-m from October 2015 as a result of the data collected between February and September 2015 indicating that fluxes may change further up the tree stems. Tree stem CH_4 fluxes at 2.00-m ($p < 0.05$, $r^2 = 0.193$, $\chi^2 = 5.764$; Fig. 2.a) varied significantly during the four months they were sampled.

Tree stem CH_4 fluxes

Tree stem fluxes varied spatially, from mostly positive at 0.30-m to mostly negative at 2.00-m, suggesting that the lower portions of tree stems may be sources of CH_4 whilst negligible emissions or uptake may the norm for tree stems over 2.00-m. There was a significant difference in stem fluxes between species at 2.00-m ($p < 0.01$, $r^2 = 0.193$ $\chi^2 = 8.184$; Fig. 4). Median fluxes at 0.30-m and 2.00-m were similar over the sampling period, whereas Ash fluxes were larger than Sycamore fluxes at 0.75-m and 1.30-m (Table 1).

Stem height (m)	**Flux range (µg m^{-2} hr^{-1})**		**Median flux (µg m^{-2} hr^{-1})**	
	Ash	Sycamore	Ash	Sycamore
0.30	-30 – 59.6	-26.7 – 37.1	12.71	12.92
0.75	-32.9 – 35.8	-52.1 – 29.6	7.50	4.58
1.30	-29.2 – 29.6	-62.1 – 37.1	-8.96	2.50
2.00	-54.2 - 61.3	-43.8 – 28.3	-17.9	-15.8

Table 1. Range and median of CH_4 fluxes recorded at 0.30-m, 0.75-m, 1.30-m and 2.00-m from Ash and Sycamore trees in Wytham Woods between October 2015 and January 2016.

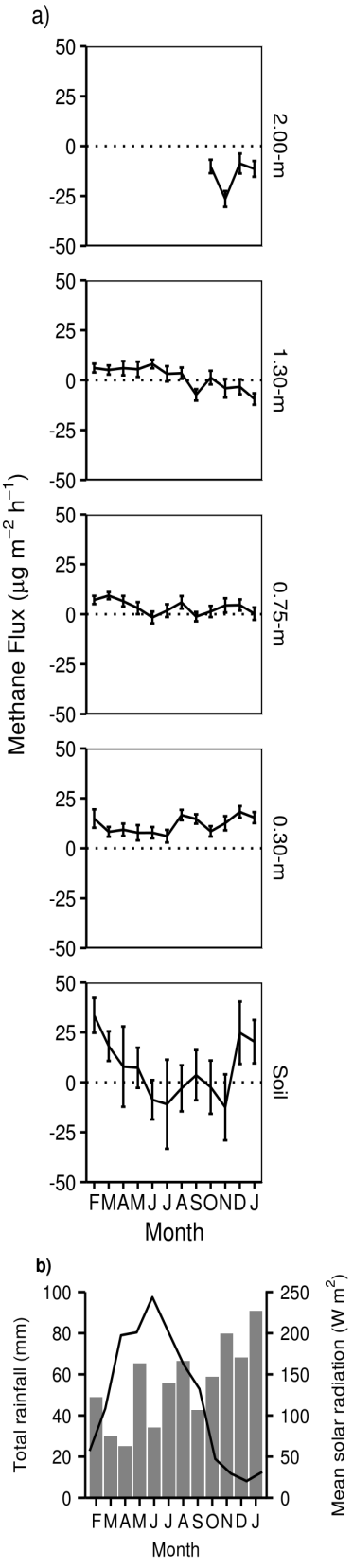

Figure 2. a) Seasonal patterns of methane (CH_4) fluxes from tree stems and soil in a temperate woodland on free-draining soil in Oxfordshire, UK, showing monthly mean stem fluxes , measured at 0.30-m, 0.75-m, 1.30-m and 2.00-m stem height and monthly mean soil fluxes measured over chambers between February 2015 and January 2016; error bars show the standard error means for *n* = 4; b) bars show the total monthly rainfall and the line shows mean solar radiation at Wytham Woods. Stem CH_4 fluxes are pooled from both species.

Ash and Sycamore stem fluxes at 0.30-m are generally positive, mean Ash stem flux was 12±2.70 µg m^{-2} hr^{-1} compared to 13.1±1.53 µg m^{-2} hr^{-1} from Sycamore stems (Fig. 3). At 0.75-m Ash stem median fluxes are consistently positive whereas as Sycamore stem median fluxes are highly variable, particularly between July and November 2015, the variation leading to a mean Ash flux of 4.56±1.89 µg m^{-2} hr^{-1}, substantially more that of Sycamore 0.19±2.44 µg m^{-2} hr^{-1}.

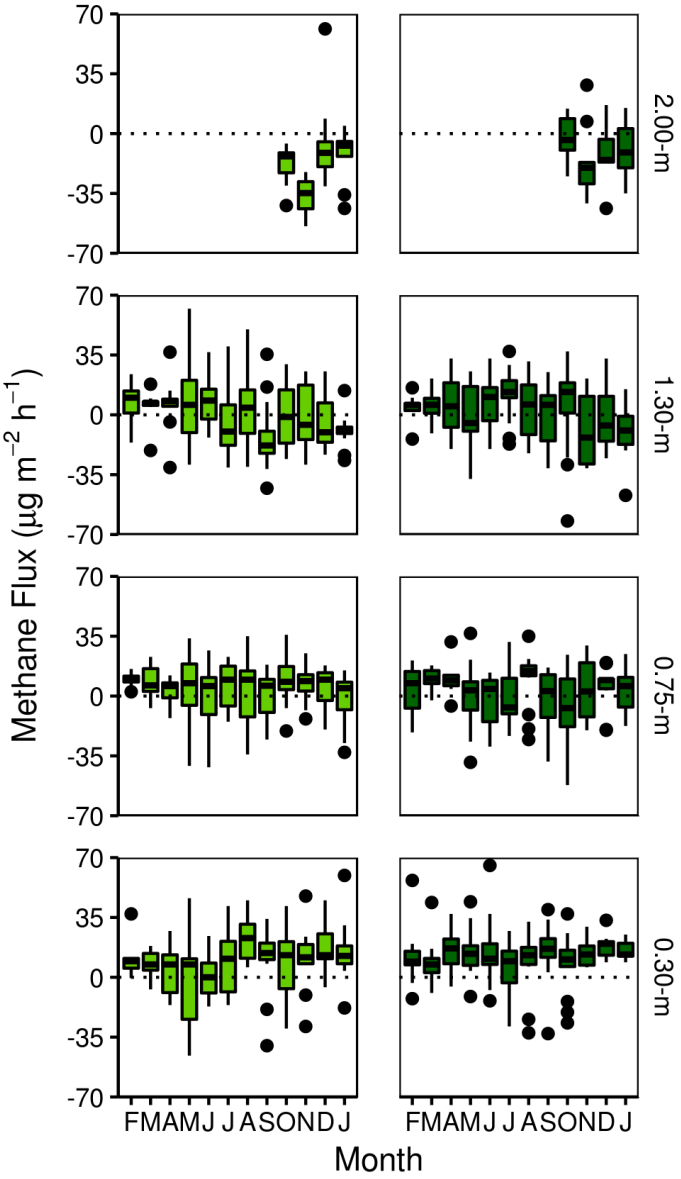

Figure 3. Monthly ranges of CH_4 fluxes from tree stems in a temperate forest on free-draining soil in Oxfordshire, UK, showing stem fluxes measured at 0.30-m, 0.75-m and 1.30-m in two common species: Ash (left panels) and Sycamore (right panels) between February 2015 and January 2016.

Mean fluxes at 1.30-m were -3.82±2.19 µg m^{-2} hr^{-1} and -2.26±3.25 µg m^{-2} hr^{-1} for Ash and Sycamore respectively. At 2.00-m fluxes were mostly negative, mean Ash stem flux was

-18.5±3.31 μg m^{-2} hr^{-1} and Sycamore mean stem flux was significantly ($p < 0.01$, $r^2 = 0.193$, $\chi^2 = 8.18$) less negative at -10.7±2.78 μg m^{-2} hr^{-1}.

Controls of soil and stem CH_4 fluxes

Between October 2015 and January 2016, stem fluxes at 0.3-m had marginally significant positive relationship with soil temperature ($p < 0.1$, $r^2 = 0.059$, $\chi^2 = 3.81$; Fig. 4). At 1.3-m CH_4 tree stem fluxes at Wytham Woods were increased by the interaction between soil temperature × air temperature ($p < 0.05$, $r^2 = 0.249$, $\chi^2 = 7.59$). There no significant relationships between air temperature, soil temperature, SWC or solar radiation and stem fluxes at 2.00-m.

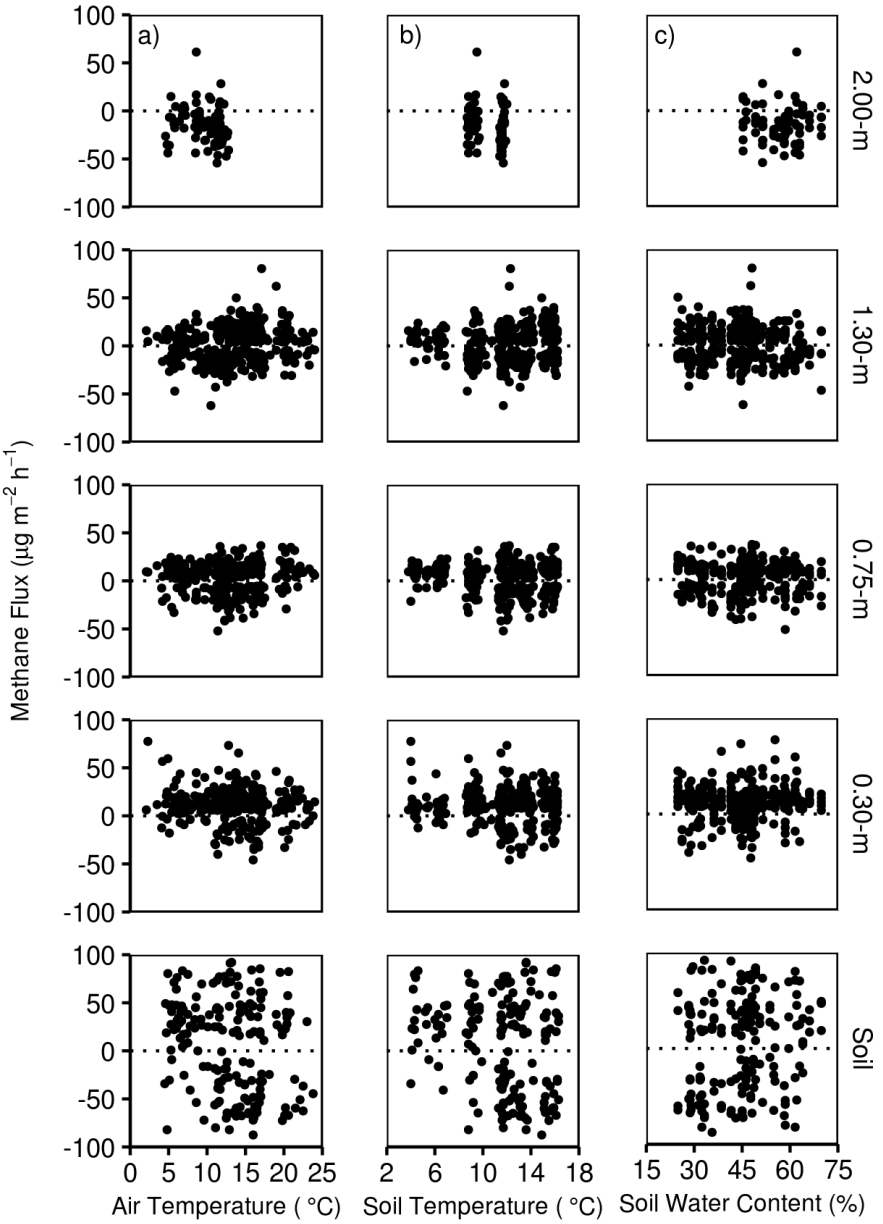

Figure 4. Scatter plots of the relationship between CH_4 fluxes from tree stems and soil chambers and a) air temperature, b) soil temperature and c) soil water content (SWC) in a temperate forest on free-draining soil in Oxfordshire, UK. Tree stem CH_4 fluxes shown are pooled between Ash and Sycamore.

Appendix III: Chapter 4 results including outliers

Seasonal variation in CH_4 fluxes

There was no clear seasonal pattern in soil chamber CH_4 fluxes; although they tended to be lower in the summer, they were not significantly so (Fig. 1.a). Soil CH_4 fluxes decreased significantly with soil temperature ($p < 0.0001$, $r^2 = 0.042$, $\chi^2 = 12.2$; Fig. 3) and there was also a significant interaction between soil temperature × SWC ($p < 0.01$, $r^2 = 0.042$, $\chi^2 = 9.90$).

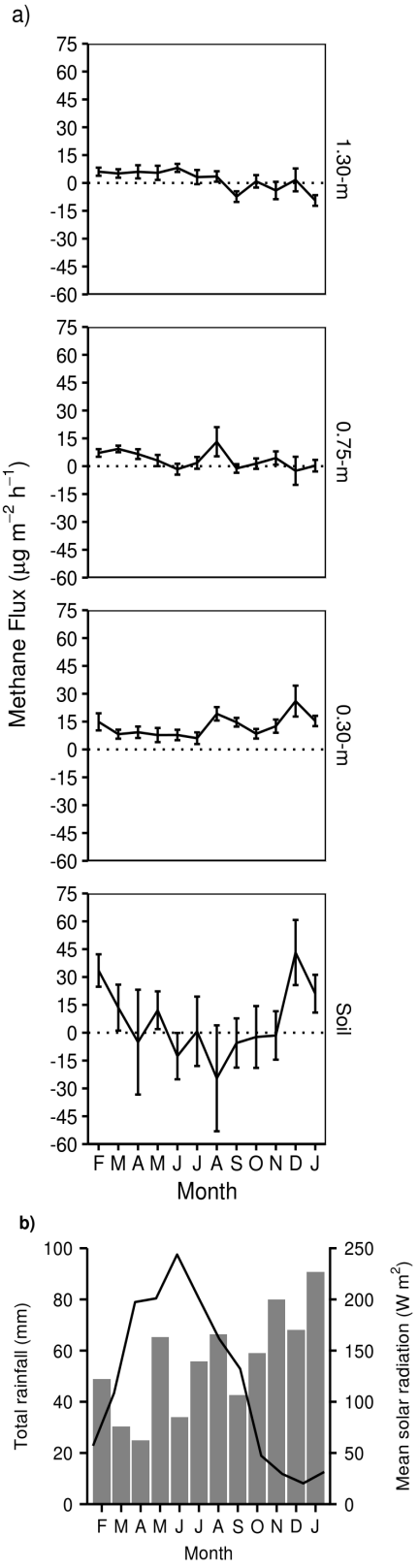

Figure 1. a) Seasonal patterns of methane (CH_4) fluxes from tree stems and soil in a temperate woodland on free-draining soil in Oxfordshire, UK, showing monthly mean stem fluxes, measured at 0.3-m, 0.75-m, 1.3-m and 2-m and monthly mean soil fluxes measured over chambers between February 2015 and January 2016; error bars show the standard error means for n = 4; b) bars show the total monthly rainfall and the line shows mean solar radiation at Wytham Woods. Tree stem CH_4 fluxes shown are the mean of Ash and Sycamore across all the plots.

No clear seasonal trend was identified at the four stem CH_4 sampling heights. Tree stem CH_4 fluxes were not related to air temperature, soil temperature, SWC or total monthly rainfall but CH_4 fluxes declined significantly with sampling height ($p < 0.0001$, $r^2 = 0.100$, $\chi^2 = 97.5$).

Stem CH_4 fluxes were positively related to solar radiation at 1.3-m ($p < 0.05$, $r^2 = 0.055$, $\chi^2 = 4.87$) and had a marginally negative relationship at 0.3-m ($p < 0.1$, $r^2 = 0.134$, $\chi^2 = 3.64$). At 1.3-m height, there was a marginally significant solar radiation × air temperature interaction ($p < 0.05$, $r^2 = 0.136$, $\chi^2 = 4.89$).

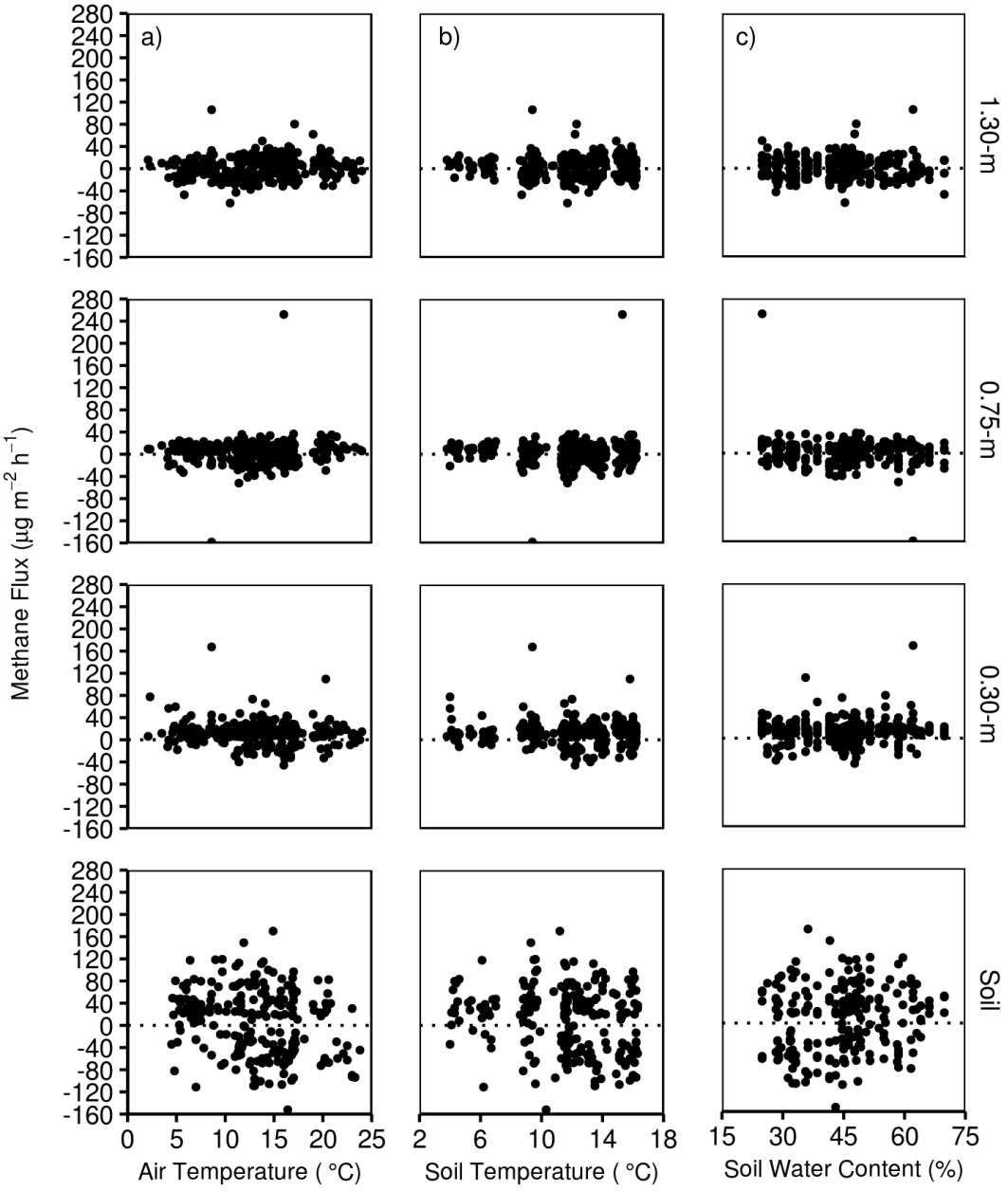

Figure 2. Scatter plots of the relationship between methane (CH_4) fluxes from soil chambers or tree stems at 0.3-m, 0.75-m, 1.3-m and 2-m sampling height and a) air temperature, b) soil temperature and c) soil water content (SWC) in a temperate woodland on free-draining soil in Oxfordshire, UK between February 2015 and January 2016. Tree stem CH_4 fluxes shown are pooled fluxes from both species across all sampling plots.

Soil CH_4 flux

Soil chamber CH_4 fluxes were negative for much of the year, declining from emission to uptake in the spring and summer before transitioning back to emission in the winter months of December (Fig. 3) and January, which coincided with the highest mean SWC values. The median soil CH_4 flux between February 2015 and January 2016 was 19.2 µg m^{-2} hr^{-1} and fluxes ranged from -625 µg m^{-2} hr^{-1} to 282 µg m^{-2} hr^{-1}. Unlike tree stem CH_4 fluxes there was no significant or marginal change over time. Overall the soils were a small source between February 2015 and January 2016 with a mean flux of 2.88±5.20 µg m^{-2} hr^{-1}.

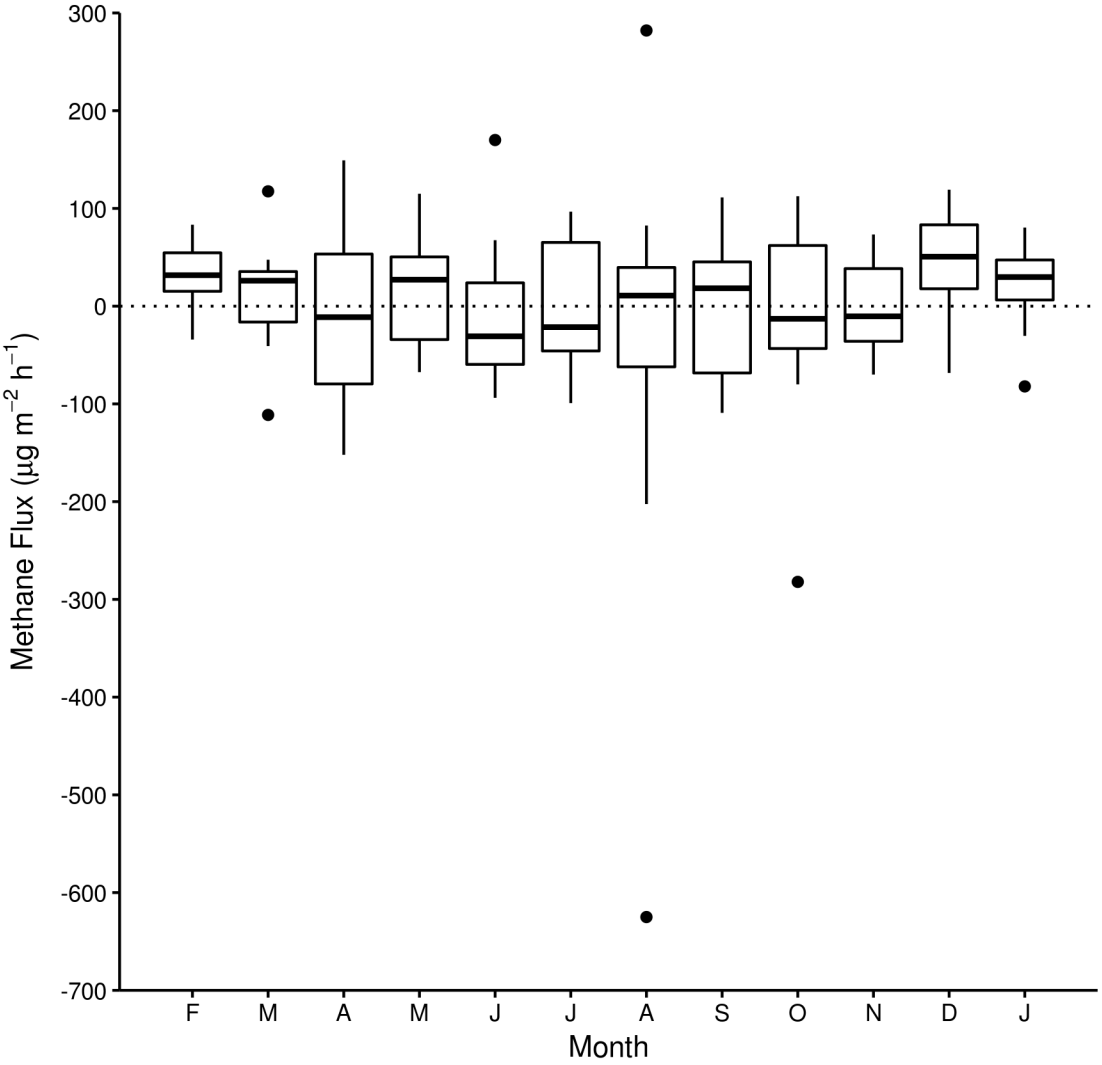

Figure 3. Monthly ranges of methane (CH_4) fluxes measured over soil chambers in a temperate woodland on free-draining soil in Oxfordshire, UK, between February 2015 and January 2016. Ranges represent four replicates.

Tree stem CH_4 fluxes

Tree stem CH_4 fluxes varied with sampling height, from mostly positive at 0.3-m to mostly negative at 1.3-m. Ash stem CH_4 fluxes were significantly lower than Sycamore stem CH_4 fluxes at 1.3-m ($p < 0.05$, $r^2 = 0.142$, $\chi^2 = 3.87$). The range of CH_4 fluxes was greater from Sycamore stems at each height (Table 1).

Stem height (m)	**Flux range (µg m^{-2} hr^{-1})**		**Median flux (µg m^{-2} hr^{-1})**	
	Ash	Sycamore	Ash	Sycamore
0.3	-45.8 – 110	-32.9 – 168	11.3	12.9
0.75	-41.7 – 35.8	-158 – 252	7.50	6.67
1.3	-42.9 – 62.1	-62.1 – 106	4.17	5.63
2	-54.2 – 61.3	-142 – 283	-17.9	-16.3

Table 1. Range and median of CH_4 fluxes recorded at 0.3-m, 0.75-m, 1.3-m and 2-m from Ash and Sycamore trees in Wytham Woods between February 2015 and January 2016.

Ash and Sycamore stem CH_4 fluxes at 0.3-m were generally positive. Mean CH_4 flux was 10.4 ±1.59 µg m^{-2} hr^{-1} from Ash stems compared to 13.7 ±1.39 µg m^{-2} hr^{-1} from Sycamore stems (Fig. 4). At 0.75-m, Ash stem median CH_4 fluxes were consistently positive whereas median Sycamore stem CH_4 fluxes were highly variable, particularly between July and November 2015. As a result, mean CH_4 flux from Ash stems at 0.75-m height was 3.99±1.15 µg m^{-2} hr^{-1}, which was almost that of Sycamore (2.43±2.21 µg m^{-2} hr^{-1}). At 1.3-m, both species showed a trend of declining CH_4 fluxes over the sampling period, which was more pronounced in Ash. At this height the mean CH_4 flux from ash stems was very slightly negative (-0.37±1.35 µg m^{-2} hr^{-1}) whereas mean CH_4 flux from Sycamore stems was 3.85±1.35 µg m^{-2} hr^{-1}.

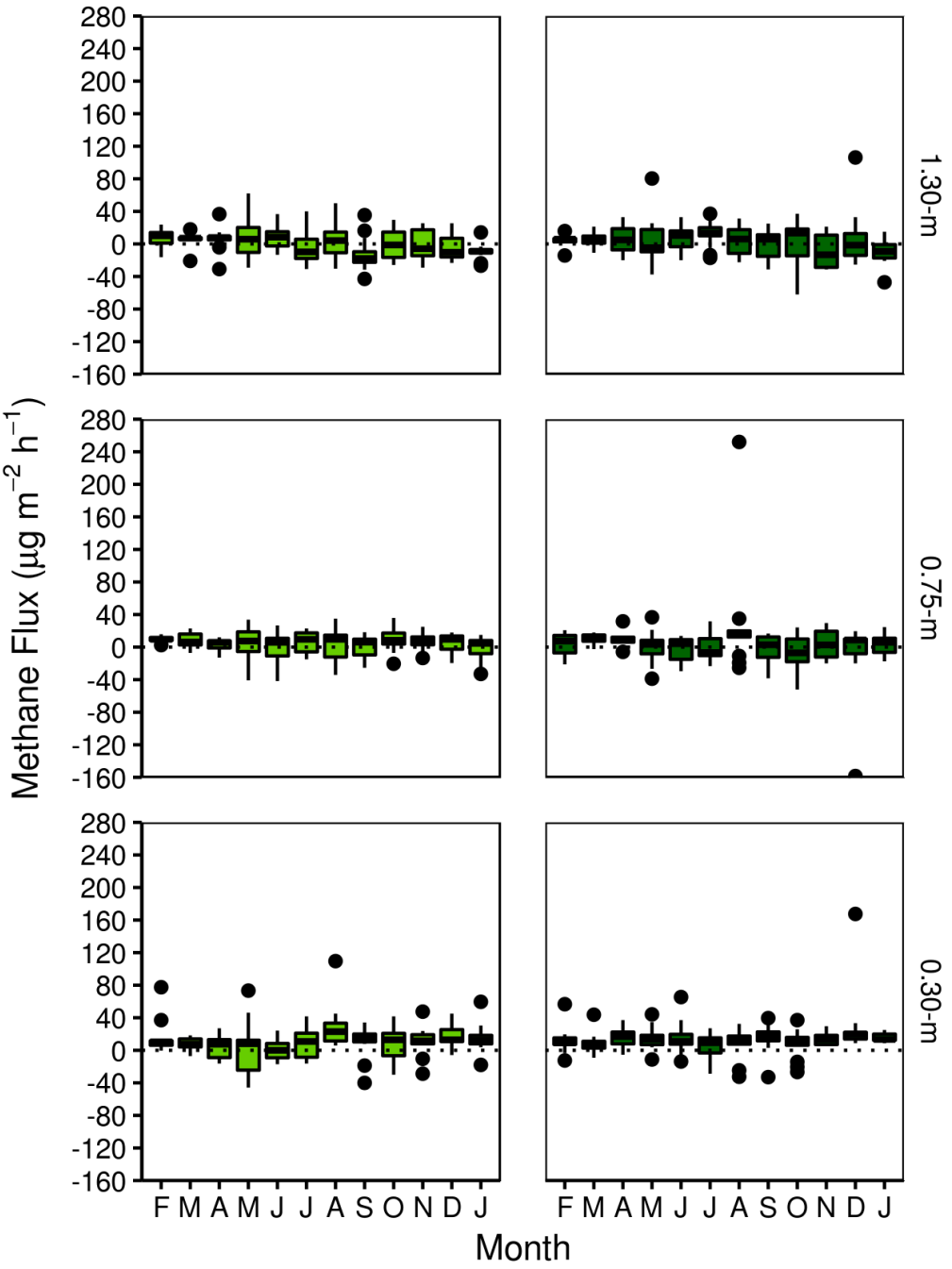

Figure 4. Monthly ranges of methane (CH_4) fluxes from tree stems in a temperate woodland on free-draining soil in Oxfordshire, UK, showing stem fluxes measured at 0.3-m, 0.75-m, 1.3-m and 2-m in two common species: Ash (pale green, left panels) and Sycamore (dark green, right panels) between February 2015 and January 2016. Ranges are based on four replicates per species.

Seasonal variation in N_2O flux

Although soil N_2O fluxes tended to be higher during months with high rainfall (Fig. 4.6.a), there were no clear seasonal patterns in soil N_2O fluxes, nor was there a significant difference between seasons. There was no significant relationship between soil N_2O fluxes and any of the measured climatic variables.

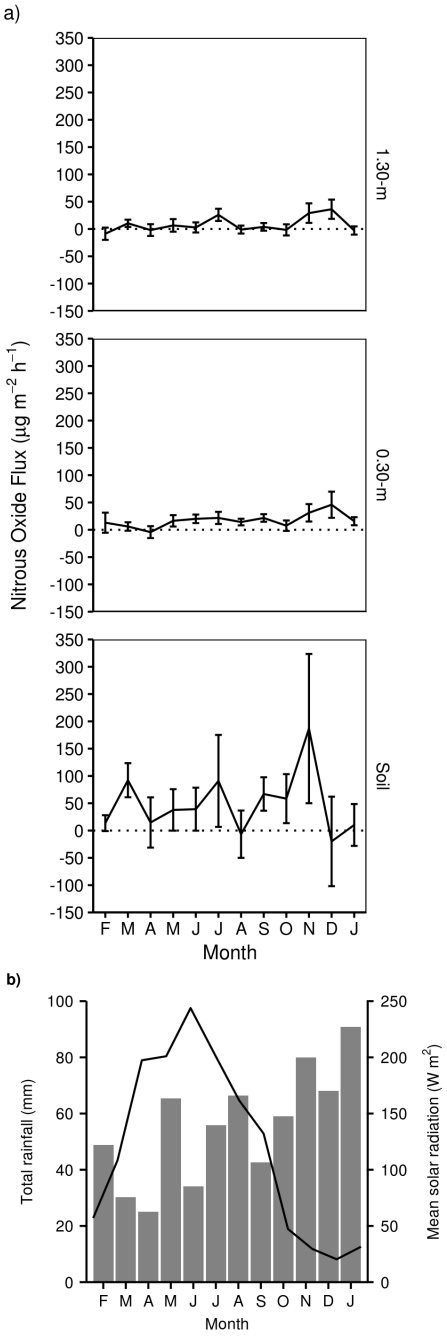

Figure 5. a) Seasonal patterns of nitrous oxide (N_2O) fluxes from tree stems and soil in a temperate woodland on free-draining soil in Oxfordshire, UK, showing monthly mean stem fluxes , measured at 0.3-m and 1.3-m, and monthly mean soil fluxes measured over chambers between February 2015 and January 2016; error bars show the standard error means for n = 4; b) bars show the total monthly rainfall and the line shows mean solar

radiation at Wytham Woods. Tree stem N_2O fluxes are the mean of pooled Ash and Sycamore stem N_2O fluxes from all sampling plots.

Similarly, there was no seasonal pattern for tree stem N_2O fluxes (Fig. 5.a) nor were there any significant effects of species, air temperature, soil temperature or soil water content.

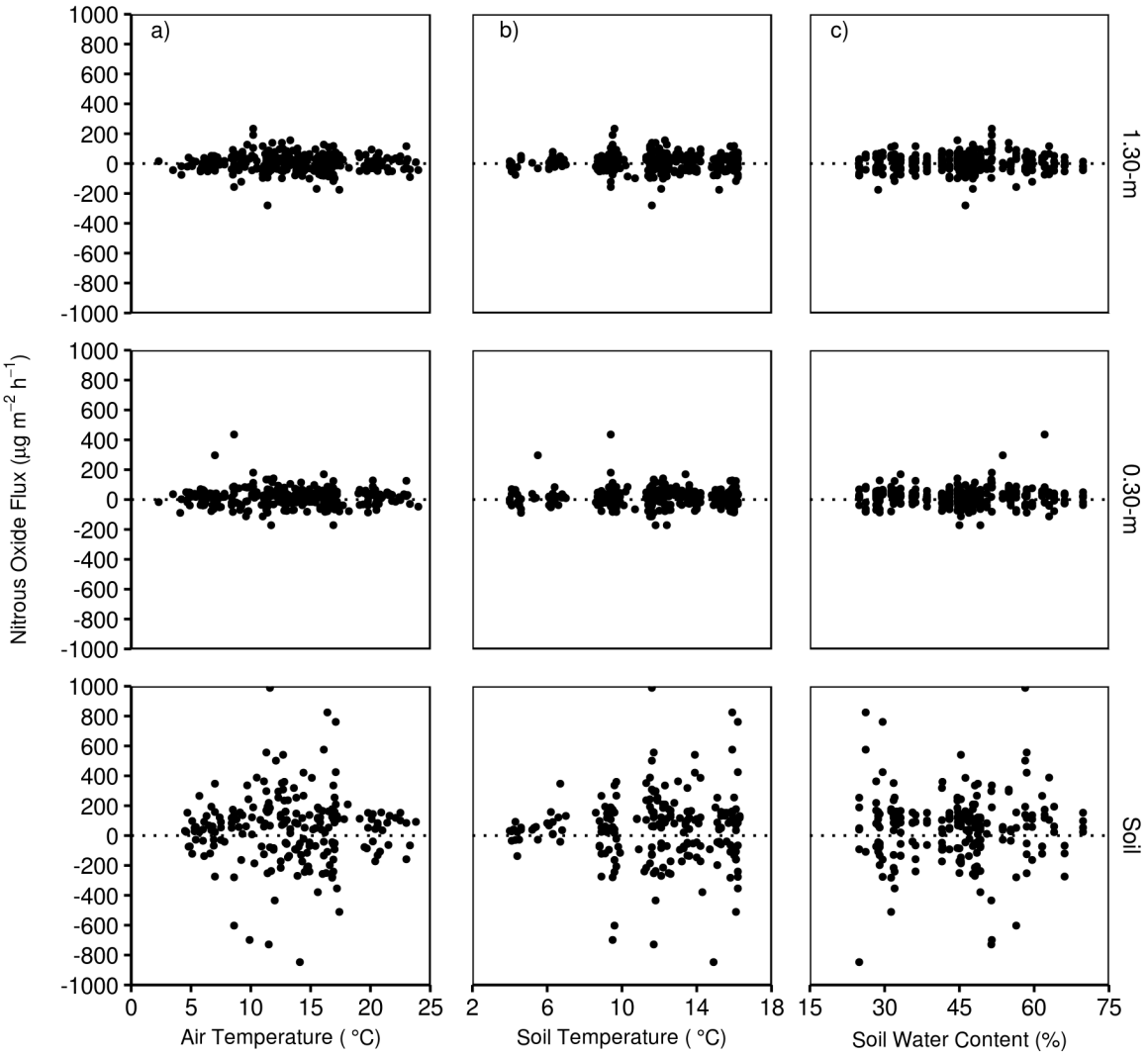

Figure 6. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from soil chambers or tree stems at 0.3-m and 1.3-m and a) air temperature, b) soil temperature and c) soil water content (SWC) in a temperate woodland on free-draining soil in Oxfordshire, UK between February 2015 and January 2016. Tree stem N_2O fluxes are pooled from Ash and Sycamore stems from all sampling plots.

Soil N_2O fluxes

Soil N_2O fluxes were mostly positive throughout the study, with the majority of uptake between April and October. The sudden increase in N_2O fluxes in November coincided with increased rainfall at Wytham Woods (Fig. 4.6). Over the course of the year N_2O fluxes ranged from -847 μg m^{-2} hr^{-1} to 989 μg m^{-2} hr^{-1} with a median flux of 60 μg m^{-2} hr^{-1}. The mean soil N_2O flux over the 12-month study was 45±15.8 μg m^{-2} hr^{-1}.

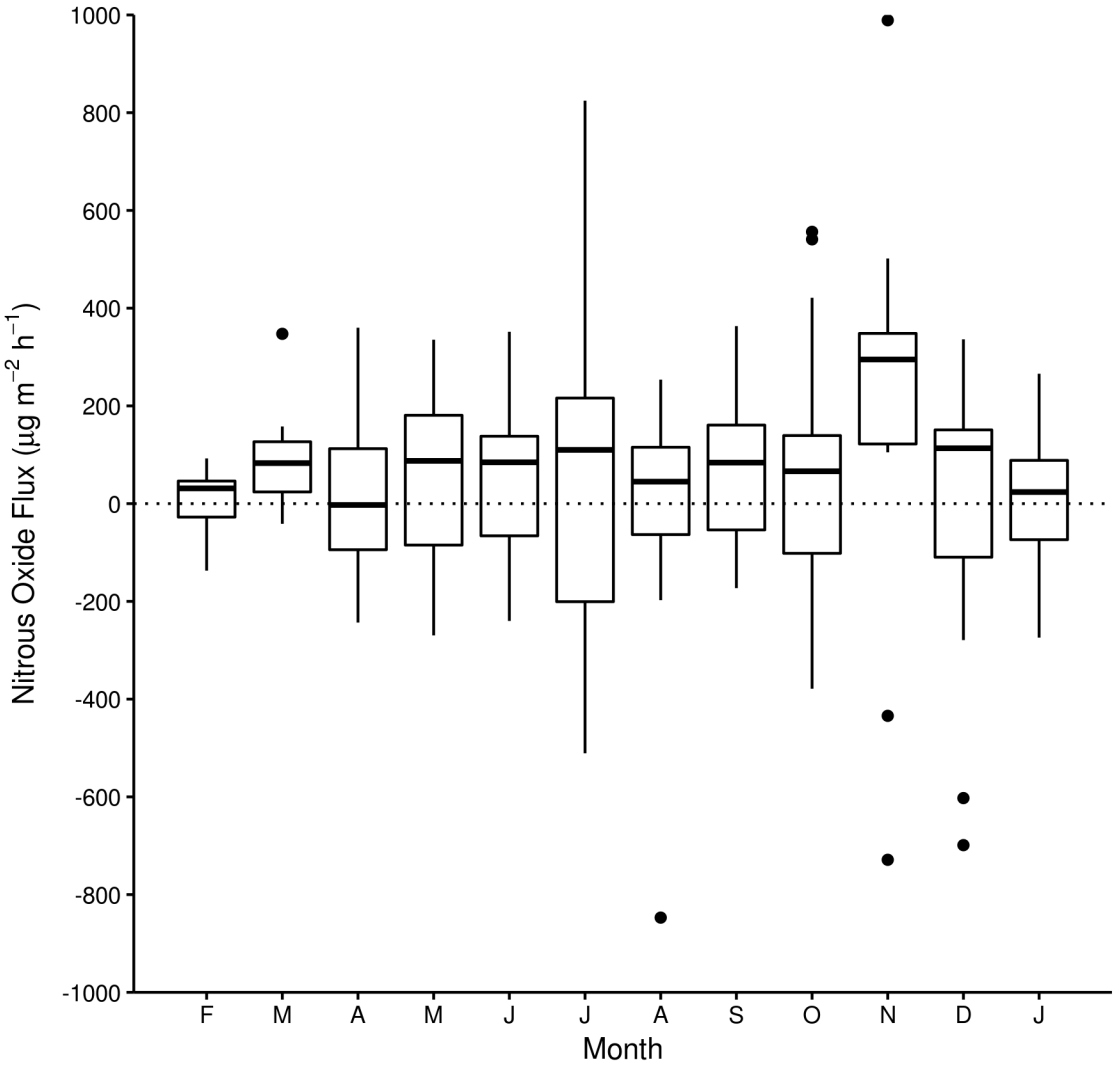

Figure 7. Monthly ranges of nitrous oxide (N_2O) fluxes measured over soil chambers in a temperate woodland on free-draining soil in Oxfordshire, UK, between February 2015 and January 2016. Ranges are based on four replicates.

Tree stem N_2O fluxes

Similar to the pattern observed for CH_4 fluxes, tree stem N_2O fluxes also decreased with stem height ($p < 0.05$, $r^2 = 0.037$, $\chi^2 = 4.37$) but there was no difference between species at either 0.3-m or 1.3-m sampling height. The range of N_2O fluxes at each sampling height was smaller for Ash stems than for Sycamore (Table 2).

Stem height (m)	**Flux range (µg m^{-2} hr^{-1})**		**Annual median flux (µg m^{-2} hr^{-1})**	
	Ash	Sycamore	Ash	Sycamore
0.3	-172 – 180	-171 – 436	20.6	25.4
1.3	-157 – 127	-280 – 234	15.8	12.1

Table 2. Range and median of N_2O fluxes recorded at 0.3-m and 1.3-m from Ash and Sycamore trees in Wytham Woods between February 2015 and January 2016.

At 0.3-m sampling height, stem fluxes of N_2O were generally positive, although the median N_2O flux for both species was negative in April 2015 (Fig. 4.9). Mean stem N_2O fluxes at 0.3-m were 13.1 ±4.10 µg m^{-2} hr^{-1} for Ash and 20.9 ±4.99 µg m^{-2} hr^{-1} for Sycamore. Stem N_2O fluxes at 1.3-m stem height were a lot more variable throughout the sampling period. At 1.3-m, mean stem N_2O flux was 12.1 ±3.93 µg m^{-2} hr^{-1} for ash and 3.02 ±5.16 µg m^{-2} hr^{-1} for Sycamore.

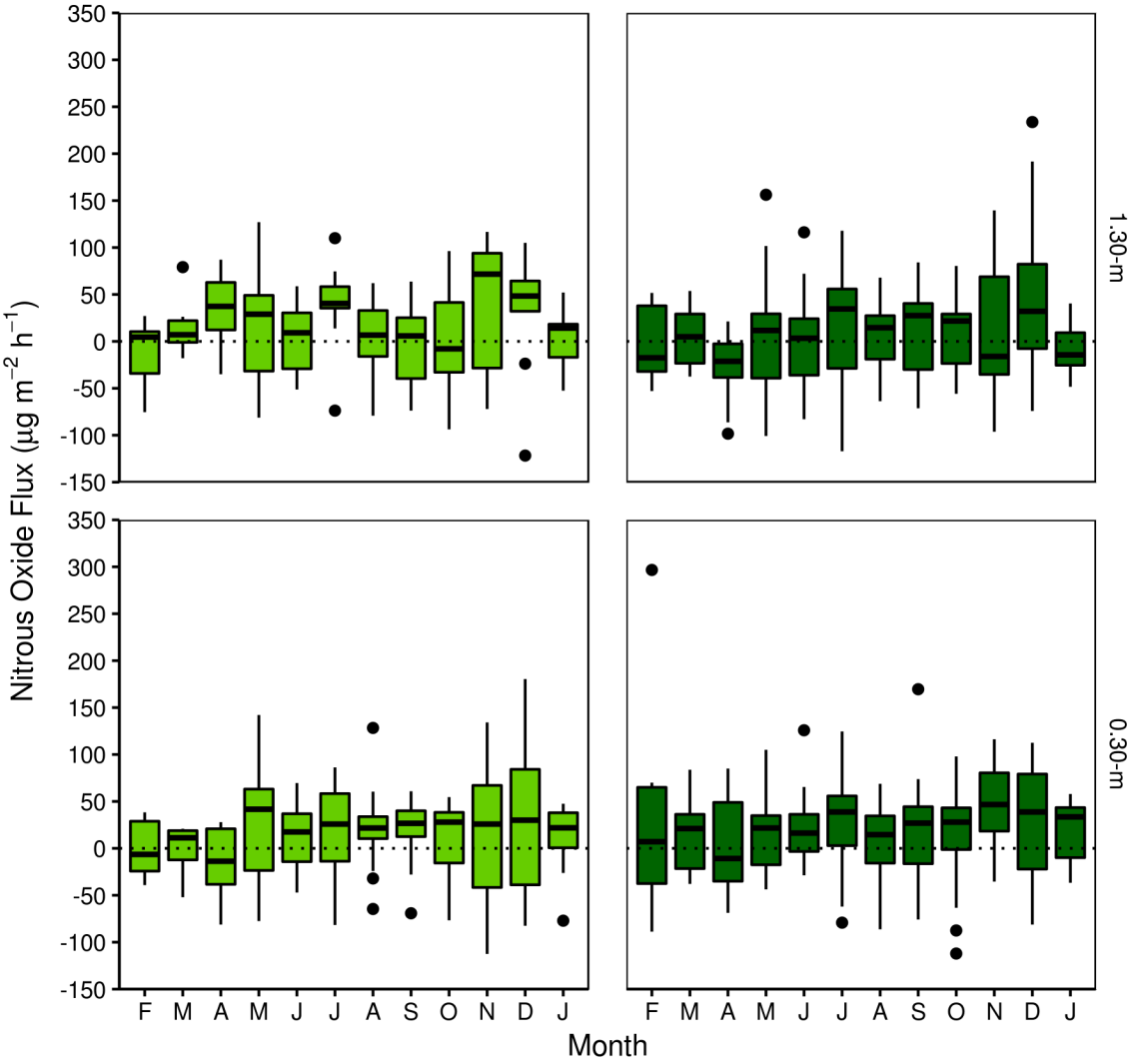

Figure 8. Monthly ranges of nitrous oxide (N_2O) fluxes from tree stems in a temperate woodland on free-draining soil in Oxfordshire, UK, showing stem fluxes measured at 0.3-m and 1.3-m in two common species: Ash (pale green, left panels) and Sycamore (dark green, right panels) between February 2015 and January 2016. Ranges are based on four replicates per species.

Appendix IV – Nitrous oxide fluxes from a lowland tropical rainforest including outliers

1. N_2O fluxes (outliers removed)

Tree stem N_2O fluxes decreased significantly with height ($p < 0.05$, $r^2 = 0.053$, $\chi^2 = 4.26$) overall. However N_2O fluxes decreased more profoundly from *Simarouba amara*, with the species × height interaction being significant ($p < 0.05$, $r^2 = 0.060$, $\chi^2 = 4.41$; Fig. 1).

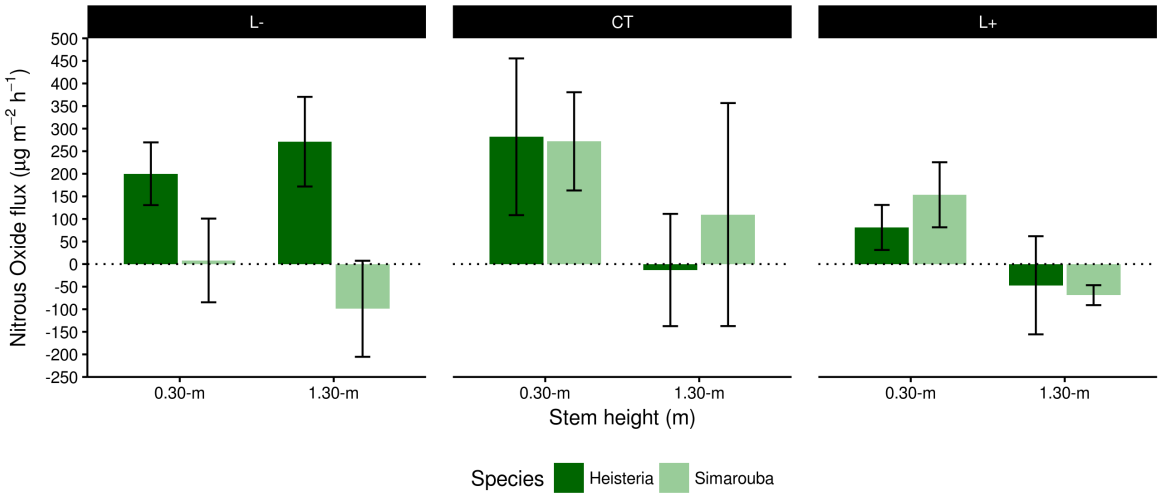

Figure 1. Bar plot of mean nitrous oxide (N_2O) fluxes against sampling height measured from stems of two common tree species: Heisteria concinna (dark green) and Simarouba amara (light green) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015; error bars show the standard error means for *n* = 4. Means are based on four replicates per species.

N_2O fluxes at 0.3-m had a marginal negative correlation between the interaction of soil temperature × rainfall ($p < 0.1$, $r^2 = 0.254$, $\chi^2 = 5.35$; Fig. 2.b). Tree stem N_2O fluxes at 0.3-m were marginally related to species × litter treatment ($p < 0.1$, $r^2 = 0.304$, $\chi^2 = 11$), whereby *Heisteria* N_2O fluxes were greater than those from *Simarouba* in litter removal plots.

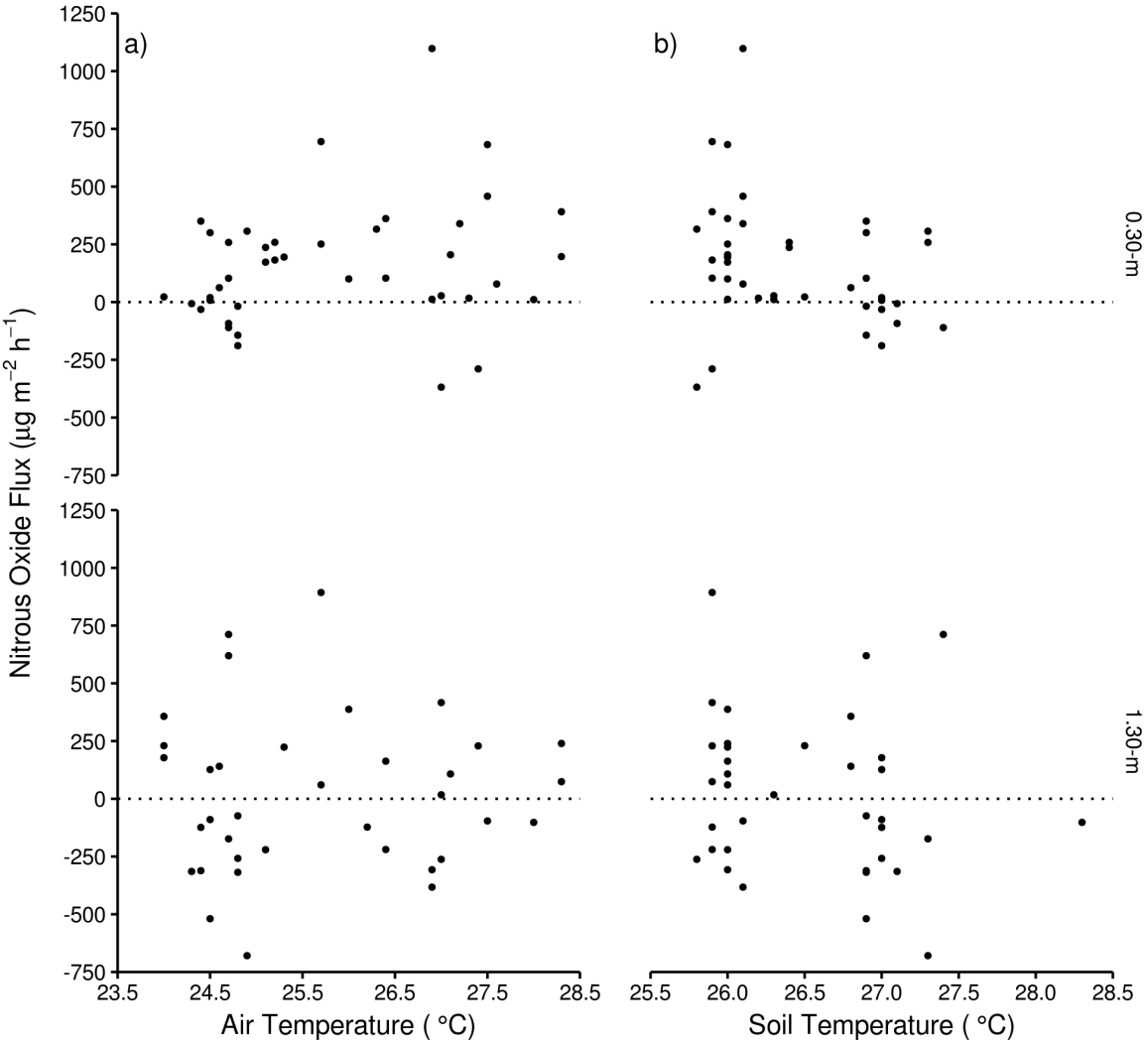

Figure 2. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems at 0.3-m and 1.3-m and a) air temperature and b) soil temperature in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015. Tree stem N_2O fluxes shown are pooled fluxes measured from *Heisteria concinna* and *Simarouba amara* and across all litter treatments.

2. N_2O fluxes (including outliers)

Tree stem N_2O fluxes decreased significantly with sampling height ($p < 0.05$, $r^2 = 0.052$, $\chi^2 = 4.30$; Fig. 3). The decrease in stem N_2O fluxes was marginally affected by the interaction of species × sampling height ($p < 0.1$, $r^2 = 0.060$, $\chi^2 = 3.17$).

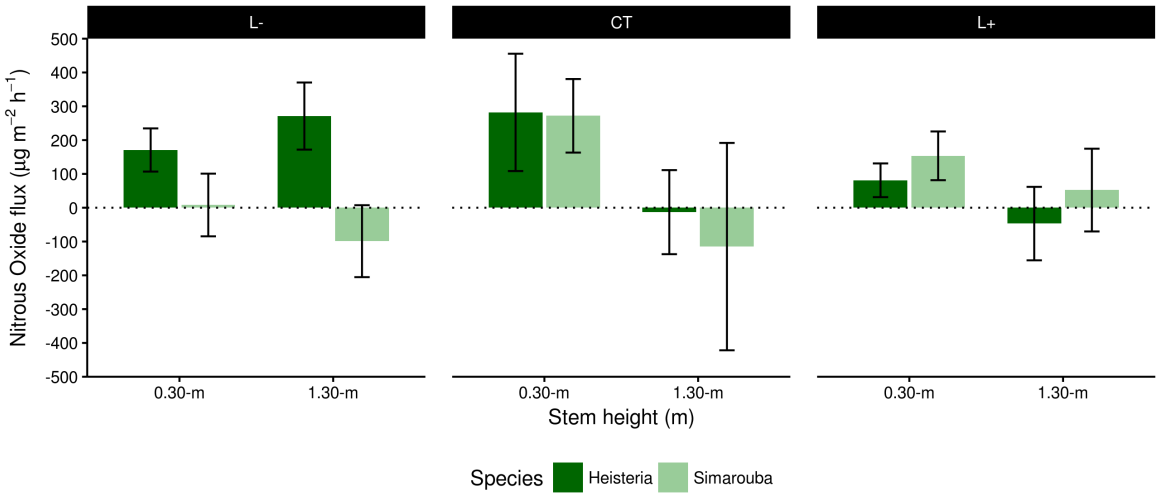

Figure 3. Bar plot of mean nitrous oxide (N_2O) fluxes against sampling height measured from stems of two common tree species: Heisteria concinna (dark green) and Simarouba amara (light green) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015; error bars show the standard error means for *n* = 4. Means are based on four replicates per species.

N_2O fluxes measured at 0.3-m significantly decreased with soil temperature ($p < 0.05$, $r^2 = 0.383$, $\chi^2 = 4.56$; Fig. 4.b) however no other climatic variables had significant or marginal effects on stem N_2O fluxes at that height. There were no effects of species, treatment or their interaction on stem N_2O flux. No significant effects of abiotic or biotic variables were found for tree stem N_2O fluxes at 1.3-m.

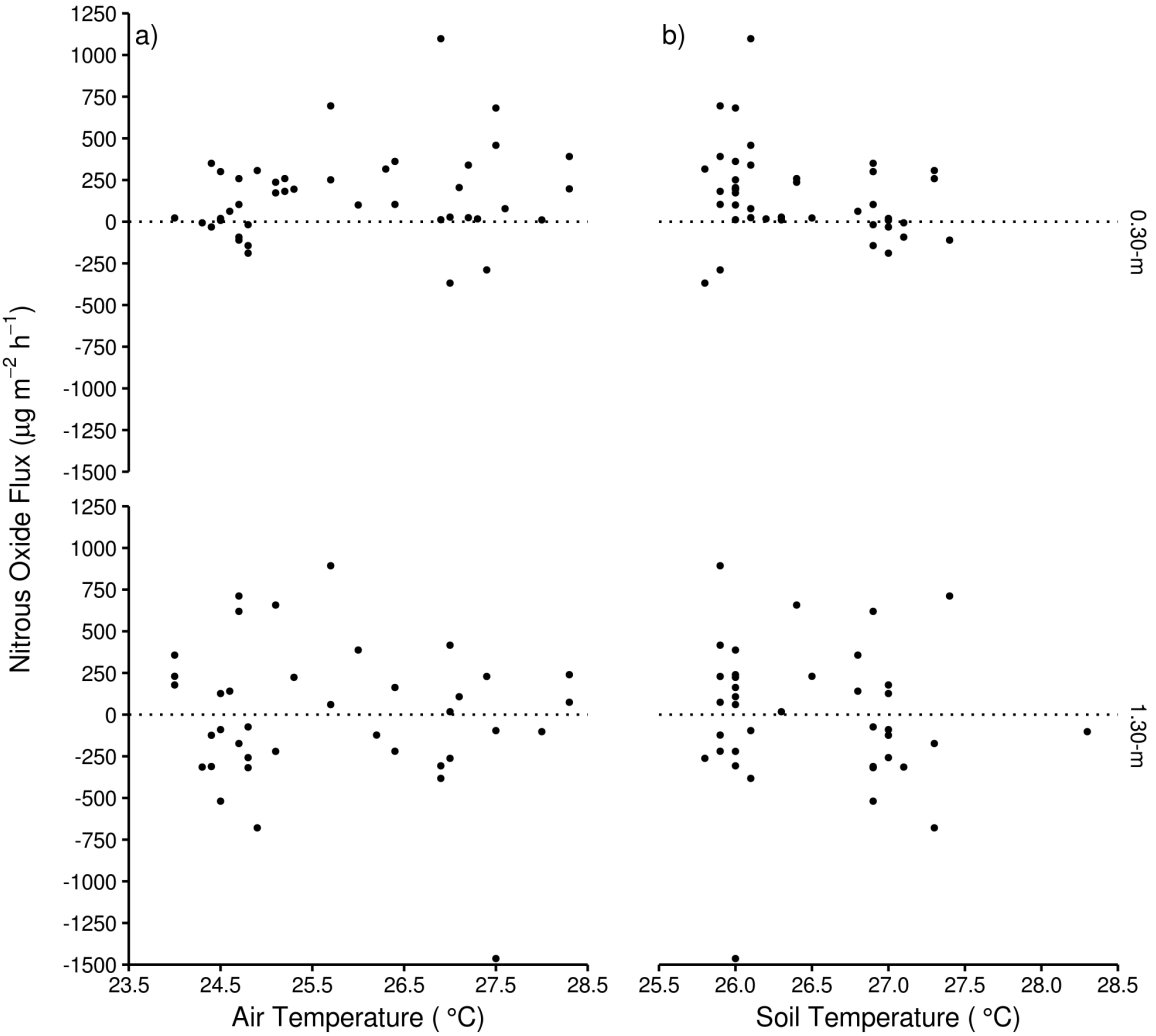

Figure 4. Scatter plots of the relationship between nitrous oxide (N_2O) fluxes from tree stems at 0.3-m and 1.3-m and a) air temperature and b) soil temperature in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015. Tree stem N_2O fluxes shown are pooled fluxes measured from *Heisteria concinna* and *Simarouba amara* and across all litter treatments.

Appendix V – Spatial variation of stem CH_4 fluxes and pivot points including outliers

1. Spatial variation and abiotic controls of stem CH_4 fluxes

Tree stem fluxes decreased significantly with sampling height in Panama ($p < 0.0001$, $r^2 = 0.052$, $\chi^2 = 29.4$; Fig. 1). Fluxes of CH_4 measured at 0.3-m and 0.75-m sampling heights were positive on average, with CH_4 fluxes at 1.3-m and 2-m stem height negative on average. Tree stem CH_4 fluxes at 0.3-m were marginally greater from *Heisteria* stems ($p < 0.1$, $r^2 = 0.320$, $\chi^2 = 6.94$). Stem CH_4 at 0.75-m were significantly affected by the treatment × species interaction ($p < 0.01$, $r^2 = 0.477$, $\chi^2 = 16.1$), where stem CH_4 fluxes from *Heisteria* stems were largely positive in litter addition plots whereas *Simarouba* stem CH_4 fluxes in the same plots were mostly negative. Stem CH_4 fluxes in Panama were largely unaffected by abiotic climate factors in addition to litter manipulation.

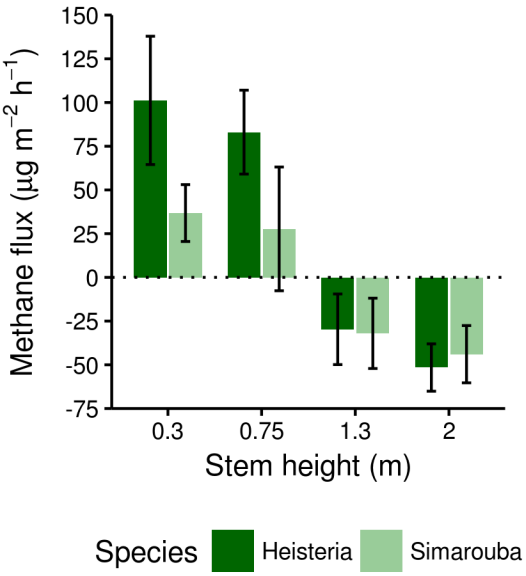

Figure 1. Bar plot of mean methane (CH_4) fluxes against sampling height measured from stems of two common tree species: *Heisteria concinna* (dark green) and *Simarouba amara* (light green) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America, between 18th and 27th November 2015; error bars show the standard error of the means for $n = 4$. Means are based on four replicates per species. Fluxes are the means of pooled fluxes from all litter manipulation plots.

In the UK, at Wytham Woods, stem fluxes also decreased significantly with sampling height ($p < 0.0001$, $r^2 = 0.168$, $\chi^2 = 70.8$; Fig. 2).There was a marginally significant difference between stem CH_4 fluxes between Ash and Sycamores at 0.75-m ($p < 0.1$, $r^2 = 0.229$, $\chi^2 = 2.91$). When outliers were included in the analyses, CH_4 were not significantly affected by abiotic factors. Tree stem CH_4 fluxes were marginally higher with increased SWC at 0.3-m (SWC × Soil

temperature interaction $p < 0.1$, $r^2 = 0.073$, $\chi^2 = 4.76$). Abiotic factors had no significant effect on tree stem CH_4 fluxes at 0.75-m. For tree stem fluxes of CH_4 at 1.3-m in Wytham Woods had a marginally positive relationship with soil temperature ($p < 0.1$, $r^2 = 0.135$, $\chi^2 = 3.75$). At 2-m no relationships were found between stem CH_4 and abiotic factors.

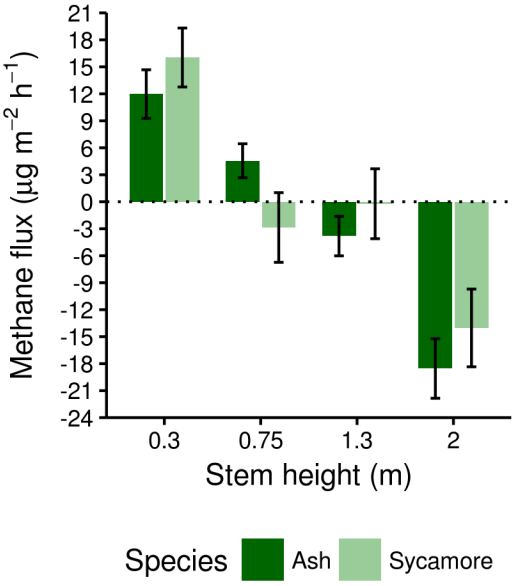

Figure 2. Bar plot of mean methane (CH_4) fluxes against sampling height measured from stems of two common tree species: Ash (dark green) and Sycamore (light green) in a temperate deciduous woodland on free-draining soil in Oxfordshire, UK, between October 2015 and January 2016; error bars show the standard error means for $n = 4$. Means are based on four replicates per species.

2. Pivot Points

In Panama the median pivot point (the height at which stem fluxes equal 0 µg CH_4 m^{-2} hr^{-1}) height for across all litter manipulation treatments was 1.59-m for *Heisteria concinna* and 1.12-m for *Simarouba amara* (Fig. 3.a). Pivot point heights for *Heisteria* ranged from 0.63-2.22-m, with a mean pivot point height of 1.40±0.19-m. Pivot points ranged from 0-3.60-m on *Simarouba* stems with a mean pivot point height of 1.49±0.35-m. There was no effect of tree species, litter treatment or the species × treatment interaction. Nor were there effects of abiotic controls on pivot point heights.

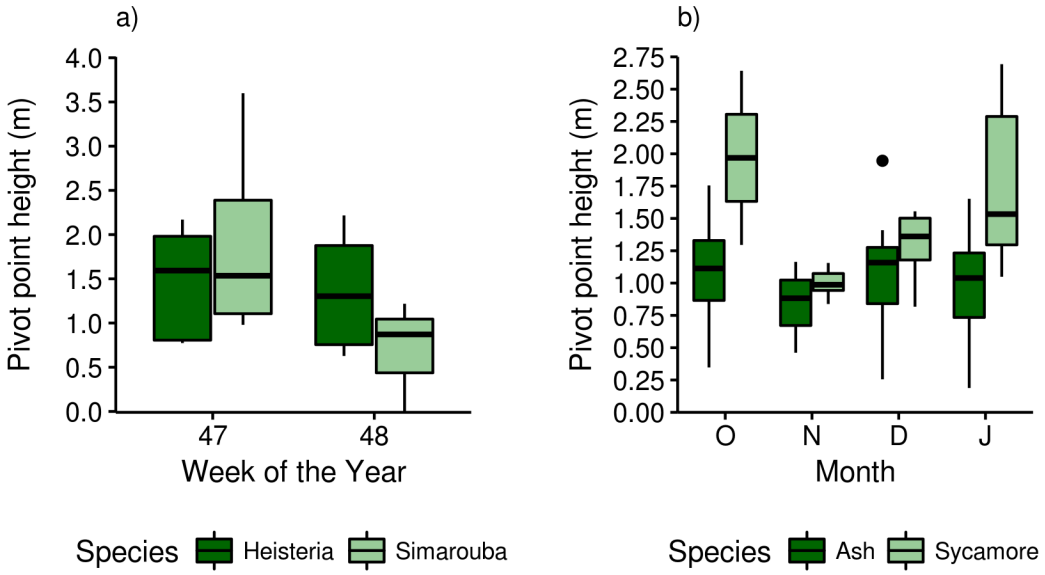

Figure 3. Ranges of pivot points from a) two common tropical tree species: *Heisteria concinna* (dark green) and *Simarouba amara* (light green) in experimental litter removal (L-), control (CT) and litter addition (L+) treatments in a lowland tropical forest on free-draining soil in Panama, Central America between 18th November and 27th November 2015; b) two common temperate forest species: Ash and Sycamore on free-draining soils in Oxfordshire, UK, between October 2015 and January 2016. Ranges are based on four replicates per species.

At the UK site, the median pivot point height for Ash trees was 1.12-m and 1.18-m for Sycamores. Pivot point heights on Ash trees ranged from 0.19-6.23-m for Ash trees and Sycamore pivot point heights ranged from 0.82-2.69-m (Fig. 3.b). Mean Ash pivot point height was 1.25±0.25-m and 1.38±0.13-m was the mean pivot point height on Sycamore stems. There was no significant difference between the two species in pivot point heights.

Appendix VI – Estimated monthly ecosystem CH_4 flux contributions at Wytham Woods

Season	Tree stem CH_4 (g ha^{-1} d^{-1})	Soil CH_4 (g ha^{-2} d^{-1})
Winter	0.09±0.24	8.36±4.11
Spring	0.54±0.24	2.06±5.78
Summer	0.37±0.33	-3.57±5.59
Autumn	-0.29±0.16	-0.62±6.43

Table 1. Table of seasonal estimated tree stem CH_4 fluxes up to 2.5-m stem height and soil CH_4 fluxes in Wytham Woods, UK.

Month	Tree stem CH_4 (g ha^{-1} d^{-1})	Soil CH_4 (g ha^{-2} d^{-1})
February	0.20±0.13	8.11±3.67
March	0.36±0.10	3.22±4.66
April	0.89±0.44	-0.25±9.01
May	0.37±0.19	3.21±3.66
June	-0.14±0.14	-3.40±2.95
July	0.44±0.41	-1.74±10.36
August	0.82±0.42	-5.56±3.47
September	-0.24±0.27	-1.03±4.88
October	-0.42±0.12	-0.63±9.31
November	-0.22±0.10	-0.20±5.09
December	0.33±0.23	11.95±5.17
January	-0.25±0.37	5.00±3.50

Table 2. Table of monthly estimated tree stem CH_4 fluxes up to 2.5-m stem height and soil CH_4 fluxes in Wytham Woods, UK.

Season	Tree stem CH_4 (g ha^{-1} d^{-1})	Soil CH_4 (g ha^{-2} d^{-1})
Winter	3.96±10.25	8.36±4.11
Spring	23.14±10.14	2.06±5.78
Summer	16.02±13.76	-3.57±5.59
Autumn	-12.64±6.81	-0.62±6.43

Table 3. Table of seasonal estimated tree stem CH_4 fluxes up to 15-m stem height and soil CH_4 fluxes in Wytham Woods, UK.

Month	Tree stem CH_4 (g ha^{-1} d^{-1})	Soil CH_4 (g ha^{-2} d^{-1})
February	8.57±5.64	8.11±3.67
March	15.28±4.18	3.22±4.66
April	38.12±18.36	-0.25±9.01
May	16.04±7.89	3.21±3.66
June	-6.05±6.03	-3.40±2.95
July	18.73±17.41	-1.74±10.36
August	35.38±17.84	-5.56±3.47
September	-10.45±11.15	-1.03±4.88
October	-18.04±4.92	-0.63±9.31
November	-9.42±4.37	-0.20±5.09
December	14.03±9.47	11.95±5.17
January	-10.71±15.65	5.00±3.50

Table 4. Table of monthly estimated tree stem CH_4 fluxes up to 15-m stem height and soil CH_4 fluxes in Wytham Woods, UK.

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